UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
CHEMICAL SAFETY
AND POLLUTION
PREVENTION

July 24, 2015

MEMORANDUM

Subject: Efficacy Review for product Micro-Kill Bleach Germicidal Bleach Solution
EPA Reg. No. 37549-2;
DP Barcode: 424689

From: Marcus Rindal, Microbiologist
Efficacy Evaluation Team
Product Science Branch
Antimicrobials Division (7510P)

Thru: Mark Perry, Team Leader
Efficacy Evaluation Team
Product Science Branch
Antimicrobials Division (7510P)

MDP

To: Demson Fuller PM 32/Shrinivas Gowda
Regulatory Management Branch I
Antimicrobials Division (7510P)

Applicant: Medline Industries, Inc.
1 Medline Place
Mundelein, IL 60060

FORMULATION FROM LABEL:

Active Ingredient	% by wt.
Sodium hypochlorite	0.65%
Other Ingredients	99.35%
Total	100.00%

I BACKGROUND

The product, Micro-Kill Bleach Germicidal Bleach Solution, is a ready-to-use spray disinfectant (bactericide, fungicide, tuberculocide and virucide) for use on hard, non-porous, non-food contact surfaces in commercial, institutional, and healthcare environments. All efficacy studies were conducted at ATS Labs, located at 1285 Corporate Center Drive, Suite 110, in Eagan, MN 55121.

This data package contained EPA Form 8570-35 (Data Matrix), a proposed product label, transmittal document, a letter from the registrant's representative dated March 24, 2015, and fifty five efficacy studies (MRIDs 494865-01 through 494865-55) with Statements of No Data Confidentiality Claims for all studies embedded in each respective MRID.

II USE DIRECTIONS

TO DISINFECT

1. Always use personal protective equipment.
2. Open [Product Name] [container].
3. Replace the closure with the trigger sprayer.
4. Spray [Product Name] [bleach solution] on desired surface.
5. Scrub and wipe with towel of your choice over desired surface to be [decontaminated] [disinfected].
   {Note: one of the two statements will be on the label.} A 30 second contact time is required [for efficacy against] [to kill] [all of the] [organisms] [bacteria and viruses] on the label except a 1 minute contact time is required [for efficacy against] [to kill] [*Candida albicans*] [and] [*Trichophyton mentagrophytes*] and a 5 minutes contact time is required [for efficacy against] [to kill] [*Clostridium difficile*] [*C. difficile*] [*C. diff*] [*C DIFF*] spores. Reapply as necessary to ensure that the surface remains wet for the entire contact time.
   OR
   Allow treated surfaces to remain thoroughly wet for 5 minutes. Reapply as necessary to ensure that the surface remains wet for the entire contact time.
6. Allow surface to air dry and discard [used] towel[s] and [empty] [container] (see storage and disposal).

III AGENCY STANDARDS

Disinfectants for Use on Hard Surfaces in Hospital or Medical Environments: The effectiveness of disinfectants for use on hard surfaces in hospital or medical environments must be substantiated by data derived using the AOAC Use-Dilution Method (for water-soluble powders and liquid products) or the AOAC Germicidal Spray Products as Disinfectants Method (for spray products). Sixty carriers must be tested with each of 3 product samples, representing 3 different product lots against *Salmonella enterica* (ATCC 10708; formerly *Salmonella choleraesuis*), *Staphylococcus aureus* (ATCC 6538), and *Pseudomonas aeruginosa* (ATCC 15442). To support products labeled as "disinfectants," killing on 59 out of 60 carriers is required to provide effectiveness at the 95% confidence level.

Disinfectants for Use on Hard Surfaces in Hospital or Medical Environments (Additional Bacteria): Effectiveness of disinfectants against specific bacteria other than those named in the AOAC Use-Dilution Method, AOAC Germicidal Spray Products as Disinfectants Method, AOAC Fungicidal Test, and AOAC Tuberculocidal Activity Method, must be determined by either the

AOAC Use-Dilution Method or the AOAC Germicidal Spray Products as Disinfectants Method. Ten carriers must be tested against each specific microorganism with each of 2 product samples, representing 2 different product lots. To support products labeled as "disinfectants" for specific bacteria (other than those bacteria named in the above test methods), killing of the specific microorganism on all carriers is required.

Virucides: The effectiveness of virucides against specific viruses must be supported by efficacy data that simulates, to the extent possible in the laboratory, the conditions under which the product is intended to be used. Carrier methods that are modifications of either the AOAC Use-Dilution Method (for liquid disinfectants) or the AOAC Germicidal Spray Products as Disinfectants Method (for spray disinfectants) must be used. To simulate in-use conditions, the specific virus to be treated must be inoculated onto hard surfaces, allowed to dry, and then treated with the product according to the directions for use on the product label. One surface for each of 2 different product lots of disinfectant must be tested against a recoverable virus titer of at least 10^4 from the test surface for a specified exposure period at room temperature. Then, the virus must be assayed by an appropriate virological technique, using a minimum of four determinations per each dilution assayed. Separate studies are required for each virus. The calculated viral titers must be reported with the test results. For the data to be considered acceptable, results must demonstrate complete inactivation of the virus at all dilutions. When cytotoxicity is evident, at least a 3-log reduction in titer must be demonstrated beyond the cytotoxic level.

Disinfectants for Use as Fungicides (Against Pathogenic Fungi, Using a Modified AOAC Use-Dilution Method): The effectiveness of liquid disinfectants against specific pathogenic fungi must be supported by efficacy data using an appropriate test. The AOAC Use-Dilution Method may be modified to conform to the appropriate elements in the AOAC Fungicidal Test. The inoculum in the test must be modified to provide a concentration of at least 10^6 conidia per carrier. Ten carriers on each of 2 product samples representing 2 different product lots must be employed in the test. Killing of the specific pathogenic fungi on all carriers is required.

Note: As an interim policy, EPA is accepting studies with dried carrier counts that are at least 10^4 for *Trichophyton mentagrophytes*, *Aspergillus niger*, and *Candida albicans*. EPA recognizes laboratories are experiencing problems in maintaining dried carrier counts at the 10^6 level. This interim policy will be in effect until EPA determines that the laboratories are able to achieve consistent carrier counts at the 10^6 level.

Disinfectants for Use as Tuberculocides (Using AOAC Tuberculocidal Activity of Disinfectants Test Method): Disinfectants may bear additional label claims of effectiveness as tuberculocides when supported by appropriate tuberculocidal effectiveness data. Certain chemical classes (i.e., glutaraldehyde and quaternary ammonium compounds) are required to undergo validation testing in addition to basic testing. Products that are formulated with other chemical groups do not require validation testing. When using the existing or modified AOAC Tuberculocidal Activity Test Methods, 10 carriers for each of 2 samples, representing 2 different product lots, must be tested against *Mycobacterium bovis* BCG (a member of the *Mycobacterium tuberculosis* species complex). Killing on all carriers/slides as demonstrated in Modified Proskauer-Beck Broth, and no growth in any of the inoculated tubes of 2 additional media (i.e., Middlebrook 7H9 Broth, Kitchener's Medium) is required.

Supplemental Claims: An antimicrobial agent identified as a "one-step" disinfectant or as effective in the presence of organic soil must be tested for efficacy with an appropriate organic soil load, such as 5 percent serum. On a product label, the hard water tolerance level may differ with the level of antimicrobial activity (e.g., sanitizer vs. disinfectant) claimed. To establish efficacy

in hard water, all microorganisms (i.e., bacteria, fungi, and viruses) claimed to be controlled must be tested by the appropriate Recommended Method at the same hard water tolerance level.

Sporicidal Disinfectant against *Clostridium difficile:* The Agency has established interim guidance for the efficacy evaluation of antimicrobial products (e.g., dilutable products, ready-to-use products, spray products, towelettes) that are labeled for use to treat hard, non-porous surfaces in healthcare settings contaminated with spores of *Clostridium difficile.* The effectiveness of such a product must be substantiated by data derived from one of the following test methods: ASTM E 2414-05: Standard Test Method for Quantitative Sporicidal Three Step Method (TSM) to Determine Efficacy of Liquids, Liquid Sprays, and Vapor or Gases on Contaminated Carrier Surfaces; or ASTM E 2197: Standard Quantitative Carrier Test Method to Evaluate the Bactericidal, Fungicidal, Mycobactericidal, and Sporicidal Potencies of Liquid Chemical Germicides. Modifications to each test method will be necessary to specifically accommodate spores of *Clostridium difficile.* Because *Clostridium difficile* is an obligate anaerobe, testing should ensure adequate incubation conditions for the recovery of viable spores. The following toxigenic strains of *Clostridium difficile* may be used for testing: ATCC 43598. All products must carry a precleaning step. Results must show a minimum 6 log reduction of viable spores in 10 minutes or less. Control carrier counts must be greater than 10^6 and $<10^7$ spores/carrier.

IV Brief Description of the Data

1. MRID 494865-01, "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces," Test Organism: Rotavirus, Strain WA, for product Micro-Kill Bleach Germicidal Bleach Solution, Lot# E27S and Lot# E28S. Study conducted at ATS Labs by Mary J. Miller, M.T. Study completion date – June 23, 2014. Project Number A16785.

This study was conducted against Rotavirus, Strain WA, for product Micro-Kill Bleach Germicidal Bleach Solution, Lot# E27S and Lot# E27S. These were tested using ATS Laboratory Protocol No. MDI01051914.ROT (copy provided). The product was received as a ready to use liquid and tested as a trigger spray. On the day of use, an aliquot of stock virus was thawed and maintained at a refrigerated temperature until used in the assay. The stock virus was adjusted to contain 5% fetal bovine serum as the organic soil load. Indicator cell cultures of MA-104 (Rhesus monkey kidney) cells (ATCC CRL-2378.1) were seeded into multiwell cell culture plates and maintained at 36-38°C in a humidified atmosphere at 5-7% CO_2. Test medium used to maintain the cell cultures was Minimum Essential Medium (MEM) supplemented with, 10 µg/mL gentamicin, 100 units/mL penicillin, 2.5 µg/mL amphotericin B, 0.5 µg/mL trypsin and 2.0 mM L-glutamine. Dried virus films were prepared by spreading 200 µL of test virus inoculum uniformly over the bottoms of 3 separate 100 x 15 mm sterile glass petri dishes. These were dried at 20.0°C in a relative humidity of 40% for 20 minutes. For each lot of test substance, one dried virus film was individually exposed for 30 seconds at room temperature (21.0°C) to 4 sprays at a distance of 6-8 inches and held covered for the exposure time. Just prior to the end of the exposure time, the plates were individually scraped with a cell scraper to re-suspend the contents and at the end of the exposure time the virus-test substance mixtures were immediately passed through individual Sephadex columns utilizing the syringe plungers in order to detoxify the mixtures. The filtrates (10^{-1} dilution) were titered by 10-fold serial dilution and each dilution was then assayed for infectivity and/or cytotoxicity. The MA-104 cells in multiwell culture dishes were inoculated in quadruplicate with 100 µL of the dilutions prepared from test and control groups. The inoculum was allowed to adsorb for sixty minutes at 36-38°C in a humidified atmosphere of 5-7% CO_2, then 1.0 mL of test medium was added to each well and the cultures were incubated at 36-38°C in a humidified

atmosphere of 5-7% CO2 in sterile disposable cell culture labware. The cultures were scored periodically for 7 days for the absence or presence of CPE, cytotoxicity, and for viability. Controls included those for dried virus control, cytotoxicity, neutralization and cell viability.

2. **MRID 494865-02, "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces," Test Organism: Respiratory Syncytial Virus (RSV), Strain Long, ATCC VR-26, for product Micro-Kill Bleach Germicidal Bleach Solution, Lot# E27S and Lot# E28S. Study conducted at ATS Labs by Mary J. Miller, M.T. Study completion date – June 23, 2014. Project Number A16786.**

This study was conducted against Respiratory Syncytial Virus (ATCC VR-26, Strain Long), for product Micro-Kill Bleach Germicidal Bleach Solution, Lot# E27S and Lot# E28S. These were tested using ATS Laboratory Protocol No. MDI01051914.RSV (copy provided). The product was received as a ready to use liquid and tested as a trigger spray. On the day of use, an aliquot of stock virus was thawed and maintained at a refrigerated temperature until used in the assay. The stock virus was adjusted to contain 5% fetal bovine serum as the organic soil load. Indicator Cell cultures of Hep-2 (human larynx carcinoma) cells (ATCC CCL-23) were seeded into multiwell cell culture plates and maintained at 36-38°C in a humidified atmosphere at 5-7% CO_2. Test medium used to maintain the cell cultures was Minimum Essential Medium (MEM) supplemented with 2% (v/v) heat-inactivated fetal bovine serum (FBS), 10 µg/mL gentamicin, 100 units/mL penicillin, 2.5 µg/mL amphotericin B, and 1.0 mM L-glutamine. Dried virus films were prepared by spreading 200 µL of test virus inoculum uniformly over the bottoms of 3 separate 100 x 15 mm sterile glass petri dishes. These were dried at 20.0°C in a relative humidity of 50% for 20 minutes. For each lot of test substance, one dried virus film was individually exposed for 30 seconds at room temperature (21.0°C) to 4 sprays at a distance of 6-8 inches and held covered for the exposure time. Just prior to the end of the exposure time, the plates were individually scraped with a cell scraper to re-suspend the contents and at the end of the exposure time the virus-test substance mixtures were immediately passed through individual Sephadex columns utilizing the syringe plungers in order to detoxify the mixtures. The filtrates (10^{-1} dilution) were titered by 10-fold serial dilution and each dilution was then assayed for infectivity and/or cytotoxicity. The Hep-2 cells in multiwell culture dishes were inoculated in quadruplicate with 100 µL of the dilutions prepared from test and control groups and were incubated at 36-38°C in a humidified atmosphere of 5-7% C02 in sterile disposable cell culture labware. The cultures were scored periodically for 9 days for the absence or presence of CPE, cytotoxicity, and for viability. Controls included those for dried virus control, cytotoxicity, neutralization and cell viability.

3. **MRID 494865-03, "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces," Test Organism: Rhinovirus type 37 Strain 151-1 (ATCC VR-1147), for product Micro-Kill Bleach Germicidal Bleach Solution, Lot# E27S and Lot# E28S. Study conducted at ATS Labs by Mary J. Miller. Study completion date – June 23, 2014. Project Number A16787.**

This study was conducted against Rhinovirus type 37 (ATCC VR-1147, Strain 151-1) for product Micro-Kill Bleach Germicidal Bleach Solution, Lot# E27S and Lot# E28S. These were tested using ATS Laboratory Protocol No. MDI01051914.R37 (copy provided). The product was received as a ready to use liquid and tested as a trigger spray. On the day of use, an aliquot of stock virus was thawed and maintained at a refrigerated temperature until used in the assay. The stock virus was adjusted to contain 5% fetal bovine serum as the organic soil load. Indicator Cell cultures of MRC-5 (human embryonic lung) cells (ATCC CCL-171) were seeded into multiwell cell culture plates and maintained at 36-38°C in a humidified atmosphere at 5-7% CO_2. Test medium used to maintain the cell cultures was Minimum Essential Medium (MEM) supplemented with 10%

(v/v) heat-inactivated fetal bovine serum (FBS), 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. Dried virus films were prepared by spreading 200 µL of test virus inoculum uniformly over the bottoms of 3 separate 100 x 15 mm sterile glass petri dishes. These were dried at 15.5°C in a relative humidity of 50% for 20 minutes. For each lot of test substance, one dried virus film was individually exposed for 30 seconds at room temperature (21.0°C) to 4 sprays at a distance of 6-8 inches and held covered for the exposure time. Just prior to the end of the exposure time, the plates were individually scraped with a cell scraper to re-suspend the contents and at the end of the exposure time the virus-test substance mixtures were immediately passed through individual Sephadex columns utilizing the syringe plungers in order to detoxify the mixtures. The filtrates (10^{-1} dilution) were titered by 10-fold serial dilution and each dilution was then assayed for infectivity and/or cytotoxicity. The WI-38 cells in multiwell culture dishes were inoculated in quadruplicate with 100 µL of the dilutions prepared from test and control groups and were incubated at 31-35°C in a humidified atmosphere of 5-7% CO_2 in sterile disposable cell culture labware. The cultures were scored periodically for 7 days for the absence or presence of CPE, cytotoxicity, and for viability. Controls included those for dried virus control, cytotoxicity, neutralization and cell viability.

4. **MRID 494865-04, "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces," Test Organism: Adenovirus type 5, Strain Adenoid 75, ATCC VR-5, for product Micro-Kill Bleach Germicidal Bleach Solution, Lot# E27S and Lot# E28S. Study conducted at ATS Labs by Mary J. Miller, M.T. Study completion date – June 25, 2014. Project Number A16788.**

This study was conducted against Adenovirus type 5, ATCC VR-5, Strain Adenoid 75 for product Micro-Kill Bleach Germicidal Bleach Solution, Lot# E27S and Lot# E28S. These were tested using ATS Laboratory Protocol No. MDI01051914.ADV (copy provided). The product was received as a ready to use liquid and tested as a trigger spray. On the day of use, an aliquot of stock virus was thawed, combined and maintained at a refrigerated temperature until used in the assay. The stock virus was adjusted to contain 5% fetal bovine serum as the organic soil load. Indicator Cell cultures of A-549 (human lung carcinoma) cells (ATCC CCL-185) were seeded into multiwell cell culture plates and maintained at 36-38°C in a humidified atmosphere at 5-7% CO_2. Test medium used to maintain the cell cultures was Minimum Essential Medium (MEM) supplemented with 5% (v/v) heat-inactivated fetal bovine serum (FBS), 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. Dried virus films were prepared by spreading 200 µL of test virus inoculum uniformly over the bottoms of 3 separate 100 x 15 mm sterile glass petri dishes. These were dried at 20.0°C in a relative humidity of 40% for 20 minutes. For each lot of test substance, dried virus films were exposed for 30 seconds at room temperature (20.0°C) to 4 sprays at a distance of 6-8 inches and held covered for the exposure time. Just prior to the end of the exposure time, the plates were individually scraped with a cell scraper to re-suspend the contents and at the end of the exposure time the virus-test substance mixtures were immediately passed through individual Sephadex columns utilizing the syringe plungers in order to detoxify the mixtures. The filtrates (10^{-1} dilution) were titered by 10-fold serial dilution and each dilution was then assayed for infectivity and/or cytotoxicity. The A-549 cells in multiwell culture dishes were inoculated in quadruplicate with 100 µL of the dilutions prepared from test and control groups and were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2 in sterile disposable cell culture labware. The cultures were scored periodically for eleven days for the absence or presence of CPE, cytotoxicity, and for viability. Controls included those for dried virus control, cytotoxicity, neutralization and cell viability.

5. **MRID 494865-05, "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces," Test Organism: Poliovirus type 1, for product Micro-Kill**

Bleach Germicidal Bleach Solution, Lot# E27S and Lot# E28S. Study conducted at ATS Labs by Mary J. Miller, M.T. Study completion date – June 23, 2014. Project Number A16789.

This study was conducted against Poliovirus type 1, ATCC VR-1562, Strain Chat for product Micro-Kill Bleach Germicidal Bleach Solution, Lot# E27S and Lot# E28S. These were tested using ATS Laboratory Protocol No. MDI01051914.POL (copy provided). The product was received as a ready to use liquid and tested as a trigger spray. On the day of use, an aliquot of stock virus was thawed, combined and maintained at a refrigerated temperature until used in the assay. The stock virus was adjusted to contain 5% fetal bovine serum as the organic soil load. Indicator Cell cultures of Vero cells (ATCC CCL-81) were seeded into multiwell cell culture plates and maintained at 36-38°C in a humidified atmosphere at 5-7% CO_2. Test medium used to maintain the cell cultures was Minimum Essential Medium (MEM) supplemented with 5% (v/v) heat-inactivated fetal bovine serum (FBS), 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. Dried virus films were prepared by spreading 200 µL of test virus inoculum uniformly over the bottoms of 3 separate 100 x 15 mm sterile glass petri dishes. These were dried at 15.5°C in a relative humidity of 50% for 20 minutes. For each lot of test substance, dried virus films were exposed for 30 seconds at room temperature (20.0°C) to 4 sprays at a distance of 6-8 inches and held covered for the exposure time. Just prior to the end of the exposure time, the plates were individually scraped with a cell scraper to re-suspend the contents and at the end of the exposure time the virus-test substance mixtures were immediately passed through individual Sephadex columns utilizing the syringe plungers in order to detoxify the mixtures. The filtrates (10^{-1} dilution) were titered by 10-fold serial dilution and each dilution was then assayed for infectivity and/or cytotoxicity. The A-549 cells in multiwell culture dishes were inoculated in quadruplicate with 100 µL of the dilutions prepared from test and control groups and were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2 in sterile disposable cell culture labware. The cultures were scored periodically for seven days for the absence or presence of CPE, cytotoxicity, and for viability. Controls included those for dried virus control, cytotoxicity, neutralization and cell viability.

6. **MRID 494865-06, "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces," Test Organism: Feline Panleukopenia virus, for product Micro-Kill Bleach Germicidal Bleach Solution, Lot# E27S and Lot# E28S. Study conducted at ATS Labs by Mary J. Miller, M.T. Study completion date – July 10, 2014. Project Number A16790.**

This study was conducted against Feline Panleukopenia virus, ATCC VR-648, Strain Philips-Roxane for product Micro-Kill Bleach Germicidal Bleach Solution, Lot# E27S and Lot# E28S. These were tested using ATS Laboratory Protocol No. MDI01051914.FPLV (copy provided). The product was received as a ready to use liquid and tested as a trigger spray. On the day of use, an aliquot of stock virus was thawed, combined and maintained at a refrigerated temperature until used in the assay. The stock virus was adjusted to contain 5% fetal bovine serum as the organic soil load. Indicator Cell cultures of feline kidney cells (CRFK, ATCC CCL-94) were seeded into multiwell cell culture plates and maintained at 36-38°C in a humidified atmosphere at 5-7% CO_2. Test medium used to maintain the cell cultures was Minimum Essential Medium (MEM) supplemented with 5% (v/v) heat-inactivated fetal bovine serum (FBS), 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. Dried virus films were prepared by spreading 200 µL of test virus inoculum uniformly over the bottoms of 3 separate 100 x 15 mm sterile glass petri dishes. These were dried at 20.0°C in a relative humidity of 40% for 20 minutes. For each lot of test substance, dried virus films were exposed for 30 seconds at room temperature (21.0°C) to 4 sprays at a distance of 6-8 inches and held covered for the exposure

time. Just prior to the end of the exposure time, the plates were individually scraped with a cell scraper to re-suspend the contents and at the end of the exposure time the virus-test substance mixtures were immediately passed through individual Sephadex columns utilizing the syringe plungers in order to detoxify the mixtures. The filtrates (10^{-1} dilution) were titered by 10-fold serial dilution and each dilution was then assayed for infectivity and/or cytotoxicity. The A-549 cells in multiwell culture dishes were inoculated in quadruplicate with 100 µL of the dilutions prepared from test and control groups and were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2 in sterile disposable cell culture labware. The cultures were scored periodically for 14 days for the absence or presence of CPE, cytotoxicity, and for viability. Controls included those for dried virus control, cytotoxicity, neutralization and cell viability.

7. **MRID 494865-07, "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces," Test Organism: Hepatitis A virus, for product Micro-Kill Bleach Germicidal Bleach Solution, Lot# E27S and Lot# E28S. Study conducted at ATS Labs by Mary J. Miller, M.T. Study completion date – July 10, 2014. Project Number A16793.**

This study was conducted against Hepatitis A virus Strain HM-175, from AppTec Laboratory Services, Camden, NJ, for product Micro-Kill Bleach Germicidal Bleach Solution, Lot# E27S and Lot# E28S. These were tested using ATS Laboratory Protocol No. MDI01051914.HAV (copy provided). The product was received as a ready to use liquid and tested as a trigger spray. On the day of use, an aliquot of stock virus was thawed, combined and maintained at a refrigerated temperature until used in the assay. The stock virus was adjusted to contain 5% fetal bovine serum as the organic soil load. Indicator Cell cultures of FRhK-4 cells (fetal Rhesus monkey kidney, ATCC CRL-1688) were seeded into multiwell cell culture plates and maintained at 36-38°C in a humidified atmosphere at 5-7% CO_2. Test medium used to maintain the cell cultures was Minimum Essential Medium (MEM) supplemented with 5% (v/v) heat-inactivated fetal bovine serum (FBS), 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B and 2.0 mM L-glutamine. Dried virus films were prepared by spreading 200 µL of test virus inoculum uniformly over the bottoms of 3 separate 100 x 15 mm sterile glass petri dishes. These were dried at 20.0°C in a relative humidity of 50% for 20 minutes. For each lot of test substance, dried virus films were exposed for 30 seconds at room temperature (21.0°C) to 4 sprays at a distance of 6-8 inches and held covered for the exposure time. Just prior to the end of the exposure time, the plates were individually scraped with a cell scraper to re-suspend the contents and at the end of the exposure time the virus-test substance mixtures were immediately passed through individual Sephadex columns utilizing the syringe plungers in order to detoxify the mixtures. The filtrates (10^{-1} dilution) were titered by 10-fold serial dilution and each dilution was then assayed for infectivity and/or cytotoxicity. The A-549 cells in multiwell culture dishes were inoculated in quadruplicate with 100 µL of the dilutions prepared from test and control groups and were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2 in sterile disposable cell culture labware. The cultures were scored periodically for 14 days for the absence or presence of CPE, cytotoxicity, and for viability. Controls included those for dried virus control, cytotoxicity, neutralization and cell viability.

8. **MRID 494865-08, "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces," Test Organism: Canine Parvovirus, for product Micro-Kill Bleach Germicidal Bleach Solution, Lot# E27S and Lot# E28S. Study conducted at ATS Labs by Mary J. Miller, M.T. Study completion date – June 24, 2014. Project Number A16794.**

This study was conducted against Canine Parvovirus Strain Cornell-780916-80 (ATCC VR-2017) for product Micro-Kill Bleach Germicidal Bleach Solution, Lot# E27S and Lot# E28S. These were tested using ATS Laboratory Protocol No. MDI01051914.CPV (copy provided). The product was received as a ready to use liquid and tested as a trigger spray. On the day of use, an aliquot of stock virus was thawed, combined and maintained at a refrigerated temperature until used in the assay. The stock virus was adjusted to contain 5% fetal bovine serum as the organic soil load. Indicator Cell cultures of A-72 cells (canine tumor, ATCC CRL-1542) were seeded into multiwell cell culture plates and maintained at 36-38°C in a humidified atmosphere at 5-7% CO_2. Test medium used to maintain the cell cultures was Minimum Essential Medium (MEM) supplemented with 5% (v/v) heat-inactivated fetal bovine serum (FBS), 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. Dried virus films were prepared by spreading 200 µL of test virus inoculum uniformly over the bottoms of 3 separate 100 x 15 mm sterile glass petri dishes. These were dried at 20.0°C in a relative humidity of 40% for 20 minutes. For each lot of test substance, dried virus films were exposed for 30 seconds at room temperature (21.0°C) to 4 sprays at a distance of 6-8 inches and held covered for the exposure time. Just prior to the end of the exposure time, the plates were individually scraped with a cell scraper to re-suspend the contents and at the end of the exposure time the virus-test substance mixtures were immediately passed through individual Sephadex columns utilizing the syringe plungers in order to detoxify the mixtures. The filtrates (10^{-1} dilution) were titered by 10-fold serial dilution and each dilution was then assayed for infectivity and/or cytotoxicity. The A-549 cells in multiwell culture dishes were inoculated in quadruplicate with 100 µL of the dilutions prepared from test and control groups and were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2 in sterile disposable cell culture labware. The cultures were scored periodically for 7 days for the absence or presence of CPE, cytotoxicity, and for viability. Controls included those for dried virus control, cytotoxicity, neutralization and cell viability.

9. **MRID 494865-09, "AOAC Germicidal Spray Method," Test Organisms: *Pseudomonas aeruginosa* (ATCC 15442), *Salmonella enterica* (ATCC 10708), and *Staphylococcus aureus* (ATCC 6538). For product Micro-Kill Bleach Germicidal Bleach Solution, Lots # E27S, E28S, and E29S. Study conducted at ATS Labs by Kristen Niehaus. Study completion date – June 27, 2014. Project Number A16796.**

This study was conducted against *Pseudomonas aeruginosa* (ATCC 15442), *Salmonella enterica* (ATCC 10708), and *Staphylococcus aureus* (ATCC 6538). Three lots (Lots # E27S, E28S, and E29S) of the product, Micro-Kill Bleach Germicidal Bleach Solution, were tested using ATS Laboratory Protocol No. MDI01051514.GS.1. The product was received as a ready to use liquid and tested as a trigger spray. A 10 µL aliquot of a thawed, vortex mixed cryovial of stock culture was transferred to an initial 10 mL tube of growth medium, mixed and incubated for 24±2 hours at 35-37°C. A 10 µL aliquot of culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (1st daily transfer). Two additional daily transfers were prepared. The final test culture was incubated for 48-54 hours at 35-37°C. On the day of use, the pellicle was carefully aspirated from the *Pseudomonas aeruginosa* culture by vacuum aspiration. Each test cultures was vortex mixed (3-4 seconds) and allowed to stand ≥10 minutes prior to use. For each test culture, the upper portion was removed and pooled in a sterile vessel and mixed. For *Pseudomonas aeruginosa*, the culture was diluted by combining 3.0 mL of test organism suspension with 3.0 mL of sterile growth medium. For *Staphylococcus aureus*, the culture was diluted by combining 2.0 mL of test organism suspension with 6.0 mL of sterile growth medium. For *Salmonella enterica*, the culture was diluted by combining 3.0 mL of test organism suspension with 3.0 mL of sterile growth medium. Each final test culture was mixed thoroughly prior to use. A 0.2 mL aliquot of fetal bovine serum (FBS) was added to 3.8 mL of each prepared culture to achieve a 5% organic soil load. For the inoculation of carriers, a 10 µL aliquot of the prepared

test culture was uniformly spread over individual glass slide carriers (18 mm x 36 mm), each in a Petri dish matted with two pieces of filter paper. Inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 35.8-36.1°C and at 55.9% relative humidity. Carriers were used within 2 hours of drying. For each lot of test substance, test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface until thoroughly wet (4 sprays). The carriers were allowed to remain wet for 30 seconds at room temperature (21.0°C) and 46.2% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at identical staggered intervals to 20 mL of Letheen Broth + 0.1% sodium thiosulfate to neutralize. All subcultures were incubated for 48±2 hours at 35-37°C. Subcultures were stored at 2-8°C for one day prior to examination. Following incubation and storage, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

10. MRID 494865-10, "AOAC Tuberculocidal Activity of Disinfectant Spray Products," Test Organism: *Mycobacterium bovis* – BCG, for product Micro-Kill Bleach Germicidal Bleach Solution, Lot# E27S and Lot# E28S. Study conducted at ATS Labs by Jill Ruhme. Study completion date – August 22, 2014. Project Number A16798.

This study was conducted against *Mycobacterium bovis* – BCG, for product Micro-Kill Bleach Germicidal Bleach Solution, Lot# E27S and Lot# E28S. These were tested using ATS Laboratory Protocol No. MDI01052014.TB.1 (copy provided). The product was received as a ready to use liquid and tested as a trigger spray. A stock culture of the test organism, on 7H11 agar medium, was transferred into 20 mL tubes of Modified Proskauer-Beck Broth using ≤10 µL loopful (two 1 µL loopfuls) of culture and incubated for 20 days at 35-37°C undisturbed in a slanted position. The test culture was then transferred to a sterile tissue grinder containing 1.00 mL of 0.85% saline + 0.1% tween 80 and was macerated to break up large clumps of the test organism. A 9.0 mL aliquot of Modified Proskauer-Beck Broth was added and the suspension was transferred from the tissue grinder to a sterile test tube. After settling for 10-15 minutes, the upper portion was removed and the suspension was transferred to a sterile vessel. This culture was standardized to 20.73% Transmittance (%T) at 650 nm. A 0.10 mL aliquot of FBS was added to 1.90 mL of broth culture to yield a 5% fetal bovine serum organic soil load. Sterile 18 mm x 36 mm glass slide carriers, each in a sterile plastic Petri dish matted with two pieces of filter paper, were each inoculated with 0.01 ml (10.0 µL) of culture using a calibrated pipettor and the inoculum was spread over the surface of the slide (approximately 1 square inch). Each dish was covered and the slides were allowed to dry for 30 minutes at 35-37°C and at 40% relative humidity. The carriers were used in the test procedure within 2 hours of drying. For each lot of test substance, 10 test carriers were sprayed with the test substance at a distance of 6-8 inches from the carrier surface using 4 full sprays and were allowed to expose for 3 minutes at room temperature (21.4°C) and at 49.4% relative humidity. Following the exposure period, the excess liquid was drained off and the individual carriers were transferred at identical staggered intervals to 20 mL of Horse serum + 0.1% sodium thiosulfate to neutralize. The carrier in the neutralizer was shaken and was transferred to a vessel containing 20 mL of Modified Proskauer-Beck Broth. Within 30 minutes of neutralization, a 2.0 mL aliquot of the neutralized solution was transferred to individual vessels containing 20 mL of Middlebrook 7H9 Broth and 20 mL of Kirchner's Medium. All subculture broths were incubated at 35-37°C under aerobic conditions and were visually examined for growth following 30 and 62 day incubation periods. All plates were incubated for 21 days at 35-37°C. Following incubation, the plates were visually examined for growth and enumerated in applicable.

Controls included those for purity, sterility, visibility, initial suspension population, neutralization confirmation and carrier population.

11. **MRID 494865-11, "Fungicidal Germicidal Spray Method," Test Organism: *Candida albicans* (ATCC 10231), for product Micro-Kill Bleach Germicidal Bleach Solution, Lot# E27S and Lot# E28S. Study conducted at ATS Labs by Kristen Niehaus. Study completion date – June 27, 2014. Project Number A16800.**

This study was conducted against *Candida albicans* (ATCC 10231) for product Micro-Kill Bleach Germicidal Bleach Solution, Lot# E27S and Lot# E28S. These were tested using ATS Laboratory Protocol No. MDI01051714.FGS.3 (copy provided). The product was received as a ready to use liquid and tested as a trigger spray. From a stock culture slant of the test organism, a loopful of culture was transferred to a sufficient number of 10 mL tubes of Sabouraud Dextrose Broth and incubated for 48-54 hours at 25-30°C. The test culture was vortex mixed for 3-4 seconds and allowed to stand for ≥10 minutes and then the upper portion of the culture was removed, leaving behind any clumps or debris and was pooled into a sterile vessel and mixed. The culture was concentrated 5X by centrifugation at 3500 RPM for 15 minutes. The final test culture was mixed thoroughly prior to use. A 0.10 mL aliquot of FBS was added to 1.90 mL of prepared culture to yield a 5% fetal bovine serum organic soil load. Ten individual sterile glass slide carriers per lot (18 mm x 36 mm), each in a Petri dish matted with two pieces of filter paper, were inoculated with 10.0 µL of test organism using a calibrated pipettor. Inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 25-30°C (26.8-27.0°C) and at 65% relative humidity. Carriers were used within 2 hours of drying. For each lot of test substance, test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface using 4 full sprays. The carriers were allowed to remain wet for 1 minute at room temperature (21.66°C) and at 20.84% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at identical staggered intervals to tubes of 20 mL of subculture medium, Sabouraud Dextrose Broth + 0.07% Lecithin + 0.5% Tween 80 and 0.05% sodium thiosulfate to neutralize. Each tube was shaken thoroughly. All neutralized subcultures were incubated for 3 days at 25-30°C. Subcultures were stored at 2-8°C for 2 days prior to examination. Following incubation and storage, the subcultures were examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation and carrier population

12. **MRID 494865-12, "Fungicidal Germicidal Spray Method," Test Organism: *Trichophyton mentagrophytes*, (ATCC 9533), for product Micro-Kill Bleach Germicidal Bleach Solution, Lot# E27S and Lot# E28S. Study conducted at ATS Labs by Kristen Niehaus. Study completion date – July 2, 2014. Project Number A16801.**

This study was conducted against *Trichophyton mentagrophytes*, (ATCC 9533), for product Micro-Kill Bleach Germicidal Bleach Solution, Lot# E27S and Lot# E28S. These were tested using ATS Laboratory protocol MDI01051714.FGS.1 (copy provided). The product was received as a ready to use liquid and tested as a trigger spray. From a stock culture of the test organism, agar plates were inoculated and incubated at 25-30°C for 10 days. The mycelia were removed from sufficient plates using a sterile device and transferred to sterile glassware containing glass beads and agitated. The culture was filtered through sterile gauze to remove hyphal fragments.
The conidial concentration was estimated by counting in a hemacytometer and determined to be

2.1×10^8 conidia/mL. Fetal Bovine Serum (FBS) was added to the inoculum to yield a 5% organic soil load. Ten individual sterile glass slide carriers per lot test substance, each in a Petri dish matted with filter paper, were inoculated with 0.01 mL (10.0 µL) of test organism using a calibrated pipettor. Inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 35.9-36.0°C and at 55.7% relative humidity. Carriers were used within 2 hours of drying. Test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface using 4 pump sprays. The carriers were allowed to remain wet for 1 minute at room temperature (22.7°C) and at 43.8% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at staggered intervals to 20 mL of neutralizer. All neutralized subcultures were incubated for 10 days at 25-30°C. The agar plate subcultures were incubated for 44-76 hours at 25-30°C. Subcultures were stored at 2-8°C for two days prior to examination. Following incubation and storage, the subcultures were examined for the presence or absence of visible growth. Controls included those for sterility, neutralization confirmation, carrier counts, inoculum counts, viability, fungistasis control and confirmation of challenge fungus.

13. MRID 494865-13, "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces," Test Organism: Influenza A virus, for product Micro-Kill Bleach Germicidal Bleach Solution, Lot# E27S and Lot# E28S. Study conducted at ATS Labs by Mary J. Miller, M.T. Study completion date – June 25, 2014. Project Number A16802.

This study was conducted against Influenza A virus, Hong Kong strain (ATCC VR-544) for product Micro-Kill Bleach Germicidal Bleach Solution, Lot# E27S and Lot# E28S. These were tested using ATS Laboratory Protocol No. MDI01051914.FLUA.1 (copy provided). The product was received as a ready to use liquid and tested as a trigger spray. On the day of use, an aliquot of stock virus was thawed, combined and maintained at a refrigerated temperature until used in the assay. The stock virus was adjusted to contain 5% fetal bovine serum as the organic soil load. Indicator Cell cultures of MDCK cells (canine kidney, ATCC CCL-34) were seeded into multiwell cell culture plates and maintained at 36-38°C in a humidified atmosphere at 5-7% CO_2. Test medium used to maintain the cell cultures was Minimum Essential Medium (MEM) supplemented with 2 µg/mL TPCK-trypsin, 25 mM HEPES, 0.2% bovine serum albumin (BSA) fraction V, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. Dried virus films were prepared by spreading 200 µL of test virus inoculum uniformly over the bottoms of 3 separate 100 x 15 mm sterile glass petri dishes. These were dried at 20.0°C in a relative humidity of 40% for 20 minutes. For each lot of test substance, dried virus films were exposed for 30 seconds at room temperature (21.0°C) to 4 sprays at a distance of 6-8 inches and held covered for the exposure time. Just prior to the end of the exposure time, the plates were individually scraped with a cell scraper to re-suspend the contents and at the end of the exposure time the virus-test substance mixtures were immediately passed through individual Sephadex columns utilizing the syringe plungers in order to detoxify the mixtures. The filtrates (10^{-1} dilution) were titered by 10-fold serial dilution and each dilution was then assayed for infectivity and/or cytotoxicity. The MDCK cells in multiwell culture dishes were inoculated in quadruplicate with 100 µL of the dilutions prepared from test and control groups and were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2 in sterile disposable cell culture labware. The cultures were scored periodically for 7 days for the absence or presence of CPE, cytotoxicity, and for viability. Controls included those for dried virus control, cytotoxicity, neutralization and cell viability.

14. MRID 494865-14, "Virucidal Efficacy of a Disinfectant for Use on Inanimate

Environmental Surfaces," Test Organism: Human Immunodeficiency Virus type 1, Strain HTLV-III_B, for product Micro-Kill Bleach Germicidal Bleach Solution, Lot# E27S and Lot# E28S. Study conducted at ATS Labs by Shanen Conway, B.S. Study completion date – Nov 20, 2014. Project Number A17209.

This study was conducted against Human Immunodeficiency Virus type 1 (Strain HTLV-III_B), for product Micro-Kill Bleach Germicidal Bleach Solution, Lot# E27S and Lot# E28S. These were tested using ATS Laboratory Protocol No. MDI01051914.HIV (copy provided). The product was received as a ready to use liquid and tested as a trigger spray. On the day of use, an aliquot of stock virus was thawed and maintained at a refrigerated temperature until used in the assay. The stock virus was adjusted to contain 5% fetal bovine serum as the organic soil load. Indicator Cell Cultures consisted of cultures of MT-2 cells (human T-cell leukemia cells), obtained through the AIDS Research and Reference Reagent Program. Cultures were maintained and used in suspension in tissue culture labware at 36-38°C in a humidified atmosphere at 5-7% CO_2. Test medium used to maintain the cell cultures was RPMI-1640 supplemented with 15% (v/v) heat-inactivated FBS supplemented with 2.0 mM L-glutamine and 50 µg/mL gentamicin. Dried virus films were prepared by spreading 200 µL of test virus inoculum uniformly over the bottoms of 3 separate 100 x 15 mm sterile glass petri dishes. These were dried at 21.0°C in a relative humidity of 49.3% for 20 minutes. For each lot of test substance, one dried virus film was individually exposed for 30 seconds at room temperature (21.0°C) to 4 sprays at a distance of 6-8 inches and held covered for the exposure time. Just prior to the end of the exposure time, the plates were individually scraped with a cell scraper to re-suspend the contents and at the end of the exposure time the virus-test substance mixtures were immediately passed through individual Sephadex columns utilizing the syringe plungers in order to detoxify the mixtures. The filtrates (10^{-1} dilution) were titered by 10-fold serial dilution and each dilution was then assayed for infectivity and/or cytotoxicity. The MT-2 cells in multiwell culture dishes were inoculated in quadruplicate with 200 µL of the dilutions prepared from test and control groups and were incubated at 36-38°C in a humidified atmosphere of 5-7% C02 in sterile disposable cell culture labware. The cultures were scored periodically for 14 days for the absence or presence of CPE, cytotoxicity, and for viability. Controls included those for dried virus control, cytotoxicity, neutralization and cell viability.

15. MRID 494865-15, "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces," Test Organism: Human Coronavirus, Strain 229E, ATCC VR-740, Strain G, ATCC VR-734, for product Micro-Kill Bleach Germicidal Bleach Solution, Lot# E27S and Lot# E28S. Study conducted at ATS Labs by Mary J. Miller, M.T. Study completion date – July 10, 2014. Project Number A16804.

This study was conducted against Human Coronavirus, ATCC VR-740, Strain 229E, for product Micro-Kill Bleach Germicidal Bleach Solution, Lot# E27S and Lot# E28S. These were tested using ATS Laboratory Protocol No. MDI01051914.COR (copy provided). The product was received as a ready to use liquid and tested as a trigger spray. On the day of use, an aliquot of stock virus was thawed and maintained at a refrigerated temperature until used in the assay. The stock virus was adjusted to contain 5% fetal bovine serum as the organic soil load. Indicator cell cultures of WI-38 (human lung) cells (ATCC CCL-75) were seeded into multiwell cell culture plates and maintained at 36-38°C in a humidified atmosphere at 5-7% CO_2. Test medium used to maintain the cell cultures was Minimum Essential Medium (MEM) supplemented with 2% (v/v) heat-inactivated fetal bovine serum (FBS), 10 µg/mL gentamicin, 100 units/mL penicillin and 2.5 µg/mL amphotericin B. Dried virus films were prepared by spreading 200 µL of test virus inoculum uniformly over the bottoms of 3 separate 100 x 15 mm sterile glass petri dishes. These were dried at 20.0°C in a relative humidity of 50% for 20 minutes. For each lot of test substance, one dried virus film was individually exposed for 30 seconds at room temperature (21.0°C) to 4 sprays at a

distance of 6-8 inches and held covered for the exposure time. Just prior to the end of the exposure time, the plates were individually scraped with a cell scraper to re-suspend the contents and at the end of the exposure time the virus-test substance mixtures were immediately passed through individual Sephadex columns utilizing the syringe plungers in order to detoxify the mixtures. The filtrates (10^{-1} dilution) were titered by 10-fold serial dilution and each dilution was then assayed for infectivity and/or cytotoxicity. The WI-38 cells in multiwell culture dishes were inoculated in quadruplicate with 100 µL of the dilutions prepared from test and control groups and were incubated at 31-35°C in a humidified atmosphere of 5-7% CO_2 in sterile disposable cell culture labware. The cultures were scored periodically for 10 days for the absence or presence of CPE, cytotoxicity, and for viability. Controls included those for dried virus control, cytotoxicity, neutralization and cell viability.

16. **MRID 494865-16, "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces," Test Organism: Feline Calicivirus as a Surrogate Virus for Norovirus, for product Micro-Kill Bleach Germicidal Bleach Solution, Lot# E27S and Lot# E28S. Study conducted at ATS Labs by Shanen Conway. Study completion date – July 2, 2014. Project Number A16820.**

This study was conducted against Feline Calicivirus (F-9 strain, ATCC VR-782) as a Surrogate Virus for Norovirus for product Micro-Kill Bleach Germicidal Bleach Solution, Lot# E27S and Lot# E28S. These were tested using ATS Laboratory Protocol No. MDI01051914.FCAL (copy provided). The product was received as a ready to use liquid and tested as a trigger spray. On the day of use, an aliquot of stock virus was thawed and maintained at a refrigerated temperature until used in the assay. The stock virus was adjusted to contain 5% fetal bovine serum as the organic soil load. Indicator Cell Cultures consisted of cultures of Crandel Reese feline kidney (CRFK) cells (ATCC CCL-94). Cultures were maintained and used in suspension in tissue culture labware at 36-38°C in a humidified atmosphere at 5-7% CO_2. Test medium used to maintain the cell cultures was Minimum Essential Medium (MEM) supplemented with 5% (v/v) heat-inactivated fetal bovine serum (FBS), 10 µg/mL gentamicin, 100 units/mL penicillin and 2.5 µg/mL amphotericin B. Dried virus films were prepared by spreading 200 µL of test virus inoculum uniformly over the bottoms of separate 100 x 15 mm sterile glass petri dishes. These were dried at 20.0°C in a relative humidity of 50% for 20 minutes. For each lot of test substance, two dried virus film were individually exposed for 30 seconds at room temperature (21.0°C) to 4 sprays at a distance of 6-8 inches and held covered for the exposure time. Just prior to the end of the exposure time, the plates were individually scraped with a cell scraper to re-suspend the contents and at the end of the exposure time the virus-test substance mixtures were immediately passed through individual Sephadex columns utilizing the syringe plungers in order to detoxify the mixtures. The filtrates (10^{-1} dilution) were titered by 10-fold serial dilution and each dilution was then assayed for infectivity and/or cytotoxicity. The MT-2 cells in multiwell culture dishes were inoculated in quadruplicate with 200 µL of the dilutions prepared from test and control groups and were incubated at 31-35°C in a humidified atmosphere of 5-7% C02 in sterile disposable cell culture labware. The cultures were scored periodically for 7 days for the absence or presence of CPE, cytotoxicity, and for viability. Controls included those for dried virus control, cytotoxicity, neutralization and cell viability.

17. **MRID 494865-17, "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces," Utilizing Bovine Viral Diarrhea Virus as a Surrogate for Human Hepatitis C Virus, for product Micro-Kill Bleach Germicidal Bleach Solution, Lot# E27S and Lot# E28S. Study conducted at ATS Labs by Shanen Conway. Study completion date – July 2, 2014. Project Number A16821.**

This study was conducted against the Oregon C24v-genotype 1 strain of Bovine Viral Diarrhea Virus (BVDV) as a Surrogate for Human Hepatitis C Virus, for product Micro-Kill Bleach Germicidal Bleach Solution, Lot# E27S and Lot# E28S. These were tested using ATS Laboratory Protocol No. MDI01051914.BVD (copy provided). The product was received as a ready to use liquid and tested as a trigger spray. On the day of use, two aliquots of stock virus were thawed, combined and maintained at a refrigerated temperature until used in the assay. The stock virus contained 5% horse serum as the organic soil load. Indicator Cell cultures of bovine turbinate (BT) cells (ATCC CRL-1390), were maintained, and used at the appropriate density in tissue culture labware at 36-38°C in a humidified atmosphere at 5-7% CO_2. Test medium used to maintain the cell cultures was Minimum Essential Medium (MEM) supplemented with 5% (v/v) non-heat inactivated horse serum, supplemented with 10 µg/mL gentamicin, 100 units/mL penicillin and 2.0 µg/mL amphotericin B. Dried virus films were prepared by spreading 200 µL of test virus inoculum uniformly over the bottoms of 6 separate 100 x 15 mm sterile glass petri dishes. These were dried at 20.0°C in a relative humidity of 50% for 20 minutes. For each lot of test substance, two dried virus films were exposed for 30 seconds at room temperature (21.0°C) to 4 sprays at a distance of 6-8 inches and held covered for the exposure time. Just prior to the end of the exposure time, the plates were individually scraped with a cell scraper to re-suspend the contents and at the end of the exposure time the virus-test substance mixtures were immediately passed through individual Sephadex columns utilizing the syringe plungers in order to detoxify the mixtures. The filtrates (10^{-1} dilution) were titered by 10-fold serial dilution and each dilution was then assayed for infectivity and/or cytotoxicity. The BT cells in multiwell culture dishes were inoculated in quadruplicate with 100 µL of the dilutions prepared from test and control groups and were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2 in sterile disposable cell culture labware. The cultures were microscopically scored periodically for seven days for the absence or presence of CPE, cytotoxicity, and for viability. On the final day of incubation, a direct immunofluorescence assay (DFA) was performed using a polyclonal fluorescein conjugated antibody specific for BVDV. The DFA was performed on the 10^{-1} and 10^{-2} dilution of the test and the 10^{-4} and 10^{-5} dilutions of the dried virus control. Controls included those for input virus control, dried virus control, cytotoxicity, neutralization and cell viability.

18. MRID 494865-18, "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces," Test Organism: Influenza A (H7N9) virus (Strain wildtype A/Anhui/1/2013, CDC # 2013759189), for product Micro-Kill Bleach Germicidal Bleach Solution, Lot# E27S and Lot# E28S. Study conducted at ATS Labs by Shanen Conway, B.S. Study completion date – June 26, 2014. Project Number A16822.

This study was conducted against Influenza A (H7N9) virus (CDC # 2013759189, Strain wildtype A/Anhui/1/2013) for product Micro-Kill Bleach Germicidal Bleach Solution, Lot# E27S and Lot# E28S. These were tested using ATS Laboratory Protocol No. MDI01051914.AFLU.3 (copy provided). The product was received as a ready to use liquid and tested as a trigger spray. On the day of use, an aliquot of stock virus was thawed and maintained at a refrigerated temperature until used in the assay. The stock virus was adjusted to contain 5% fetal bovine serum as the organic soil load. Indicator Cell cultures of MDCK (canine kidney) cells (ATCC CCL-34) were seeded into multiwell cell culture plates and maintained at 36-38°C in a humidified atmosphere at 5-7% CO_2. Test medium used to maintain the cell cultures was Minimum Essential Medium (MEM) supplemented with 2 µg/mL TPCK-trypsin, 25 mM HEPES, 0.2% BSA fraction V, 10 µg/mL gentamicin, 100 units/mL penicillin and 2.5 µg/mL amphotericin B. Dried virus films were prepared by spreading 200 µL of test virus inoculum uniformly over the bottoms of 3 separate 100 x 15 mm sterile glass petri dishes. These were dried at 21.0°C in a relative humidity of 46.4% for 20 minutes. For each lot of test substance, one dried virus film was individually exposed for 1 minute at room temperature (21.0°C) to 4 sprays at a distance of 6-8 inches and held covered for

the exposure time. Just prior to the end of the exposure time, the plates were individually scraped with a cell scraper to re-suspend the contents and at the end of the exposure time the virus-test substance mixtures were immediately passed through individual Sephadex columns utilizing the syringe plungers in order to detoxify the mixtures. The filtrates (10^{-1} dilution) were titered by 10-fold serial dilution and each dilution was then assayed for infectivity and/or cytotoxicity. The MDCK cells in multiwell culture dishes were inoculated in quadruplicate with 100 µL of the dilutions prepared from test and control groups and were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2 in sterile disposable cell culture labware. The cultures were scored periodically for 6 days for the absence or presence of CPE, cytotoxicity, and for viability. Controls included those for dried virus control, cytotoxicity, neutralization and cell viability.

19. **MRID 494865-19, "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces," Test Organism: Avian Influenza A (H5N1) virus (Strain VNH5N1-PR8/CDC-RG, CDC # 2006719965), for product Micro-Kill Bleach Germicidal Bleach Solution, Lot# E27S and Lot# E28S. Study conducted at ATS Labs by Shanen Conway. Study completion date – June 26, 2014. Project Number A16823.**

This study was conducted against Avian Influenza A (H5N1) virus (Strain VNH5N1-PR8/CDC-RG, CDC # 2006719965) for product Micro-Kill Bleach Germicidal Bleach Solution, Lot# E27S and Lot# E28S. These were tested using ATS Laboratory Protocol No. MDI01051914.AFLU.2 (copy provided). The product was received as a ready to use liquid and tested as a trigger spray. On the day of use, an aliquot of stock virus was thawed and maintained at a refrigerated temperature until used in the assay. The stock virus was adjusted to contain 5% fetal bovine serum as the organic soil load. Indicator Cell cultures of MDCK (canine kidney) cells (ATCC CCL-34) were seeded into multiwell cell culture plates and maintained at 36-38°C in a humidified atmosphere at 5-7% CO_2. Test medium used to maintain the cell cultures was Minimum Essential Medium (MEM) supplemented with 2 µg/mL TPCK-trypsin, 25 mM HEPES, 0.2% BSA fraction V, 10 µg/mL gentamicin, 100 units/mL penicillin and 2.5 µg/mL amphotericin B. Dried virus films were prepared by spreading 200 µL of test virus inoculum uniformly over the bottoms of 3 separate 100 x 15 mm sterile glass petri dishes. These were dried at 21.0°C in a relative humidity of 49.1% for 20 minutes. For each lot of test substance, one dried virus film was individually exposed for 30 seconds at room temperature (21.0°C) to 4 sprays at a distance of 6-8 inches and held covered for the exposure time. Just prior to the end of the exposure time, the plates were individually scraped with a cell scraper to re-suspend the contents and at the end of the exposure time the virus-test substance mixtures were immediately passed through individual Sephadex columns utilizing the syringe plungers in order to detoxify the mixtures. The filtrates (10^{-1} dilution) were titered by 10-fold serial dilution and each dilution was then assayed for infectivity and/or cytotoxicity. The MDCK cells in multiwell culture dishes were inoculated in quadruplicate with 100 µL of the dilutions prepared from test and control groups and were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2 in sterile disposable cell culture labware. The cultures were scored periodically for 6 days for the absence or presence of CPE, cytotoxicity, and for viability. Controls included those for dried virus control, cytotoxicity, neutralization and cell viability.

20. **MRID 494865-20, "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces," Test Organism: Influenza A virus (H3N2) Reassortant virus, Strain A/Washington/897/80 x A/Mallard/New York/6750/78, ATCC VR-2072, for product Micro-Kill Bleach Germicidal Bleach Solution, Lot# E27S and Lot# E28S. Study conducted at ATS Labs by Shanen Conway, B.S. Study completion date – July 22, 2014. Project Number A16824.**

This study was conducted against Influenza A virus (H3N2) Reassortant virus, Strain A/Washington/897/80 x A/Mallard/New York/6750/78, ATCC VR-2072 for product Micro-Kill Bleach Germicidal Bleach Solution, Lot# E27S and Lot# E28S. These were tested using ATS Laboratory Protocol No. MDI01051914.AFLU.1 (copy provided). The product was received as a ready to use liquid and tested as a trigger spray. On the day of use, an aliquot of stock virus was thawed and maintained at a refrigerated temperature until used in the assay. The stock virus was adjusted to contain 5% fetal bovine serum as the organic soil load. Indicator Cell cultures of MDCK (canine kidney) cells (ATCC CCL-34) were seeded into multiwell cell culture plates and maintained at 36-38°C in a humidified atmosphere at 5-7% CO_2. Test medium used to maintain the cell cultures was Minimum Essential Medium (MEM) supplemented with 2 µg/mL TPCK-trypsin, 25 mM HEPES, 0.2% BSA fraction V, 10 µg/mL gentamicin, 100 units/mL penicillin and 2.5 µg/mL amphotericin B. Dried virus films were prepared by spreading 200 µL of test virus inoculum uniformly over the bottoms of 3 separate 100 x 15 mm sterile glass petri dishes. These were dried at 20.0°C in a relative humidity of 40% for 20 minutes. For each lot of test substance, one dried virus film was individually exposed for 30 seconds at room temperature (21.0°C) to 4 sprays at a distance of 6-8 inches and held covered for the exposure time. Just prior to the end of the exposure time, the plates were individually scraped with a cell scraper to re-suspend the contents and at the end of the exposure time the virus-test substance mixtures were immediately passed through individual Sephadex columns utilizing the syringe plungers in order to detoxify the mixtures. The filtrates (10^{-1} dilution) were titered by 10-fold serial dilution and each dilution was then assayed for infectivity and/or cytotoxicity. The MDCK cells in multiwell culture dishes were inoculated in quadruplicate with 100 µL of the dilutions prepared from test and control groups and were incubated at 36-38°C in a humidified atmosphere of 5-7% C02 in sterile disposable cell culture labware. The cultures were scored periodically for 7 days for the absence or presence of CPE, cytotoxicity, and for viability. Controls included those for dried virus control, cytotoxicity, neutralization and cell viability.

21. MRID 494865-21, "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces," Test Organism: 2009-H1N1 Influenza A virus (Novel H1N1), for product Micro-Kill Bleach Germicidal Bleach Solution, Lot# E27S and Lot# E28S. Study conducted at ATS Labs by Shanen Conway. Study completion date – July 2, 2014. Project Number A16825.

This study was conducted against 2009-H1N1 Influenza A virus (Novel H1N1), Strain A/Mexico/4108/2009 (CDC #2009712192) for product Micro-Kill Bleach Germicidal Bleach Solution, Lot# E27S and Lot# E28S. These were tested using ATS Laboratory Protocol No. MDI01051914.FLUA.2 (copy provided). The product was received as a ready to use liquid and tested as a trigger spray. On the day of use, an aliquot of stock virus was thawed and maintained at a refrigerated temperature until used in the assay. The stock virus was adjusted to contain 5% fetal bovine serum as the organic soil load. Indicator Cell cultures of MDCK (canine kidney) cells (ATCC CCL-34) were seeded into multiwell cell culture plates and maintained at 36-38°C in a humidified atmosphere at 5-7% CO_2. Test medium used to maintain the cell cultures was Minimum Essential Medium (MEM) supplemented with 2 µg/mL TPCK-trypsin, 25 mM HEPES, 0.2% BSA fraction V, 10 µg/mL gentamicin, 100 units/mL penicillin and 2.5 µg/mL amphotericin B. Dried virus films were prepared by spreading 200 µL of test virus inoculum uniformly over the bottoms of 3 separate 100 x 15 mm sterile glass petri dishes. These were dried at 21.0°C in a relative humidity of 45.0% for 20 minutes. For each lot of test substance, one dried virus film was individually exposed for 30 seconds at room temperature (21.0°C) to 4 sprays at a distance of 6-8 inches and held covered for the exposure time. Just prior to the end of the exposure time, the plates were individually scraped with a cell scraper to re-suspend the contents and at the end of

the exposure time the virus-test substance mixtures were immediately passed through individual Sephadex columns utilizing the syringe plungers in order to detoxify the mixtures. The filtrates (10^{-1} dilution) were titered by 10-fold serial dilution and each dilution was then assayed for infectivity and/or cytotoxicity. The MDCK cells in multiwell culture dishes were inoculated in quadruplicate with 100 µL of the dilutions prepared from test and control groups and were incubated at 36-38°C in a humidified atmosphere of 5-7% C02 in sterile disposable cell culture labware. The cultures were scored periodically for 7 days for the absence or presence of CPE, cytotoxicity, and for viability. Controls included those for dried virus control, cytotoxicity, neutralization and cell viability.

22. MRID 494865-22, "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces," Test Organism: Herpes simplex virus type 2, Strain G, ATCC VR-734, for product Micro-Kill Bleach Germicidal Bleach Solution, Lot# E27S and Lot# E28S. Study conducted at ATS Labs by Shanen Conway. Study completion date – July 2, 2014. Project Number A16826.

This study was conducted against Herpes simplex virus type 2, ATCC VR-734, Strain G, for product Micro-Kill Bleach Germicidal Bleach Solution, Lot# E27S and Lot# E28S. These were tested using ATS Laboratory Protocol No. MDI01051914.HSV2 (copy provided). The product was received as a ready to use liquid and tested as a trigger spray. On the day of use, an aliquot of stock virus was thawed and maintained at a refrigerated temperature until used in the assay. The stock virus contained 5% fetal bovine serum as the organic soil load. Indicator cell cultures of Rabbit kidney (RK) cells (CellPro Labs, MN) were seeded into multiwell cell culture plates and maintained at 36-38°C in a humidified atmosphere at 5-7% CO_2. Test medium used to maintain the cell cultures was Minimum Essential Medium (MEM) supplemented with 5% (v/v) heat-inactivated fetal bovine serum (FBS), 10 µg/mL gentamicin, 100 units/mL penicillin and 2.5 µg/mL amphotericin B. Dried virus films were prepared by spreading 200 µL of test virus inoculum uniformly over the bottoms of separate 100 x 15 mm sterile glass petri dishes. These were dried at 20.0°C in a relative humidity of 50% for 20 minutes. For each lot of test substance, one dried virus film was individually exposed for 30 seconds at room temperature (21.0°C) to 4 sprays at a distance of 6-8 inches and held covered for the exposure time. Just prior to the end of the exposure time, the plates were individually scraped with a cell scraper to re-suspend the contents and at the end of the exposure time the virus-test substance mixtures were immediately passed through individual Sephadex columns utilizing the syringe plungers in order to detoxify the mixtures. The filtrates (10^{-1} dilution) were titered by 10-fold serial dilution and each dilution was then assayed for infectivity and/or cytotoxicity. The RK cells in multiwell culture dishes were inoculated in quadruplicate with 100 µL of the dilutions prepared from test and control groups and were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2 in sterile disposable cell culture labware. The cultures were scored periodically for 7 days for the absence or presence of CPE, cytotoxicity, and for viability. Controls included those for dried virus control, cytotoxicity, neutralization and cell viability.

23. MRID 494865-23, "AOAC Germicidal Spray Method," Test Organism: *Shigella dysenteriae* (ATCC 11835). For product Micro-Kill Bleach Germicidal Bleach Solution, Lots #E27S and #E28S. Study conducted at ATS Labs by Mary J. Miller. Study completion date – July 10, 2014. Project Number A16828.

This study was conducted against *Shigella dysenteriae* (ATCC 11835). Two lots (Lot Nos. E27S and #E28S) of the product, Micro-Kill Bleach Germicidal Bleach Solution, were tested using ATS Laboratory Protocol No. MDI01051714.GS.11 (copy provided). The product was received as a ready to use liquid and tested as a trigger spray. A loop of stock slant culture was transferred

to an initial 10 mL tube of growth medium. The tube was mixed and the initial culture was incubated for 24±2 hours at 35-37°C. Following incubation a 10 µL aliquot of culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1). Two additional daily transfer were prepared. The final test culture was incubated for 48-54 hours at 35-37°C. The test culture was vortex mixed (3-4 seconds) and allowed to stand ≥10 minutes prior to use. The upper portion was removed and pooled in a sterile vessel and mixed. The final test culture was mixed thoroughly prior to use. A 0.1 mL aliquot of fetal bovine serum was added to 1.9 mL of prepared culture to achieve a 5% organic soil load. A 10 µL aliquot of the prepared culture was uniformly spread over 10 individual glass slide carriers (18 mm x 36 mm) per product batch, each in a Petri dish matted with two pieces of filter paper. Inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 27.0-27.2°C and at 63% relative humidity. Carriers were used within 2 hours of drying. Test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface until thoroughly wet (4 full sprays). The carriers were allowed to remain wet for 30 seconds at room temperature (20.7°C) and at 46.0% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at identical staggered intervals to 20 mL of Letheen Broth + 0.1% sodium thiosulfate to neutralize. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

24. MRID 494865-24, "AOAC Germicidal Spray Method," Test Organism: *Listeria monocytogenes* (ATCC 19117). For product Micro-Kill Bleach Germicidal Bleach Solution, Lots #E27S and #E28S. Study conducted at ATS Labs by Mary J. Miller. Study completion date – July 9, 2014. Project Number A16829.

This study was conducted against *Listeria monocytogenes* (ATCC 19117). Two lots (Lot Nos. E27S and #E28S) of the product, Micro-Kill Bleach Germicidal Bleach Solution, were tested using ATS Laboratory Protocol No. MDI01051514.GS.11 (copy provided). The product was received as a ready to use liquid and tested as a trigger spray. A loop of stock slant culture was transferred to an initial 10 mL tube of growth medium. The tube was mixed and the initial culture was incubated for 24±2 hours at 35-37°C. Following incubation a 10 µL aliquot of culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1). The final test culture was incubated for 48-54 hours at 35-37°C. The test culture was vortex mixed (3-4 seconds) and allowed to stand ≥10 minutes prior to use. The upper portion was removed and pooled in a sterile vessel and mixed. The final test culture was mixed thoroughly prior to use. A 0.1 mL aliquot of fetal bovine serum was added to 1.9 mL of prepared culture to achieve a 5% organic soil load. A 10 µL aliquot of the prepared culture was uniformly spread over the 10 individual glass slide carriers per product batch, each in a Petri dish matted with two pieces of filter paper. Inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 35-37°C (36.1°C) and at 53.9% relative humidity. Carriers were used within 2 hours of drying. Test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface until thoroughly wet (4 full sprays). The carriers were allowed to remain wet for 30 seconds at room temperature (20.4°C) and at 58.1% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at identical staggered intervals to 20 mL of Brain Heart Infusion Broth to neutralize (primary and secondary). All subcultures were incubated for 48±2 hours at 35-37°C. Following

incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

25. MRID 494865-25, "AOAC Germicidal Spray Method," Test Organism: New Delhi metallo-beta-lactamase 1 (NDM-1) positive *Klebsiella pneumoniae* (CDC 1000527) for product Micro-Kill Bleach Germicidal Bleach Solution, Lots #E27S and #E28S. Study conducted at ATS Labs by Matthew Sathe. Study completion date – June 27, 2014. Project Number A16830.

This study was conducted against New Delhi metallo-beta-lactamase 1 (NDM-1) positive *Klebsiella pneumoniae* (CDC 1000527). Two lots (Lot Nos. E27S and #E28S) of the product, Micro-Kill Bleach Germicidal Bleach Solution, were tested using ATS Laboratory Protocol No. MDI01051714.GS.7 (copy provided). The product was received as a ready to use liquid and tested as a trigger spray. A loopful of stock slant culture was transferred to an initial 10 mL tube of growth medium, mixed, and the initial culture was incubated for 24±2 hours at 35-37°C. Following incubation, a 10 µL aliquot of culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1). The final test culture was incubated for 48-54 hours at 35-37°C. The test culture was vortex mixed for 3 to 4 seconds and allowed to stand for ≥10 minutes prior to use. Then the upper portion of the culture was removed, leaving behind any clumps or debris and was pooled in a sterile vessel and mixed. The final test culture was mixed thoroughly prior to use. A 0.10 mL aliquot of FBS was added to 1.90 mL of prepared culture to yield a 5% fetal bovine serum organic soil load. Ten (10) individual sterile glass slide carriers per batch, each in a Petri dish matted with two pieces of filter paper, were inoculated with 10.0 µL of test organism using a calibrated pipettor. Inoculum was uniformly spread over the test surface (approx. 1 square inch) of the slide and covered immediately. This was repeated until all slides were individually inoculated. The culture was vortex mixed periodically during inoculation as necessary. The slides were allowed to dry for 30 minutes at 36.1°C and at 55.9% relative humidity, appeared visibly dry, and were used within 2 hours of drying. For each lot of test substance, test carriers (in an undisturbed horizontal position) were sprayed with the test substance at staggered intervals at a distance of 6-8 inches using 4 sprays, and sprayed within ±3 seconds of the exposure time following a calibrated timer. Each treated carrier was then held at room temperature (24.1°C) and 39.0% relative humidity for 30 seconds. After exposure, excess liquid was drained off the carrier without touching the carrier to the Petri dish or filter paper. Each carrier was then transferred using sterile forceps following identical staggered intervals to 20 mL aliquots of neutralizing subculture medium (Letheen Broth + 0.1% sodium thiosulfate). The vessel was shaken thoroughly. All subcultures were incubated for 48±2 hours at 35-37°C, stored at 2-8°C for 1 day, and then visually examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation and carrier population.

26. MRID 494865-26, "AOAC Germicidal Spray Method," Test Organism: Multi-Drug Resistant (MDR) *Acinetobacter baumannii* (ATCC 19606) for product Micro-Kill Bleach Germicidal Bleach Solution (Lot # E27S and Lot # E28S). Study conducted at ATS Labs by Matthew Sathe. Study completion date – July 11, 2014. Project Number A16831.

This study was conducted against Multi-Drug Resistant (MDR) *Acinetobacter baumannii* (ATCC 19606) for product Micro-Kill Bleach Germicidal Bleach Solution (Lot # E27S and Lot # E28S) using ATS Laboratory Protocol No. MDI01051514.GS.2 (copy provided). The product was received as a ready to use liquid and tested as a trigger spray. A loopful of stock slant culture was transferred to an initial 10 mL tube of growth medium (Nutrient Broth). The tube was mixed and the initial culture was incubated for 24±2 hours at 35-37°C. Following incubation a 10 µL

aliquot of culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1). The final test culture was incubated for 48-54 hours at 35-37°C. The test culture was vortex mixed for 3 to 4 seconds and allowed to stand for ≥10 minutes prior to use. After this time, the upper portion of the culture was removed, leaving behind any clumps or debris and was pooled in a sterile vessel and mixed. The culture was diluted using sterile growth medium by combining 1.00 mL of test organism suspension with 2.00 mL of sterile growth medium. The final test culture was mixed thoroughly prior to use. A 0.10 mL aliquot of FBS was added to 1.90 mL of prepared culture to yield a 5% fetal bovine serum organic soil load. Ten (10) individual sterile glass slide carriers (18 mm x 36 mm) per batch, each in a Petri dish matted with two pieces of filter paper, were inoculated with 10.0 µL of test organism using a calibrated pipettor. Inoculum was uniformly spread over the test surface (approx. 1 square inch) of the slide and covered immediately. This was repeated until all slides were individually inoculated. The culture was vortex mixed periodically during inoculation as necessary. The carriers were allowed to dry for 30 minutes at 35-37°C at 54.7% relative humidity and appeared visibly dry, and were used in the test procedure within 2 hours of drying. For each lot of test substance, test carriers (in an undisturbed horizontal position) were sprayed with the test substance at staggered intervals at a distance of 6-8 inches using 4 sprays, and sprayed within ±3 seconds of the exposure time following a calibrated timer. Following treatment, each carrier was held at room temperature (21.7°C) and 50.7% relative humidity for 30 seconds. After exposure time, excess liquid was drained off the carrier without touching the carrier to the Petri dish or filter paper. Each carrier was then transferred using sterile forceps and following identical staggered intervals to 20 mL aliquots of neutralizing subculture medium. The vessel was shaken thoroughly. All subcultures were incubated for 48±2 hours at 35-37°C, then stored at 2-8°C for 2 days, and then visually examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation and carrier population.

27. **MRID 494865-27, "AOAC Germicidal Spray Method," Test Organism: Vancomycin Resistant *Enterococcus faecalis* – VRE (ATCC 51575) for product Micro-Kill Bleach Germicidal Bleach Solution (Lot# E27S and Lot# E28S). Study conducted at ATS Labs by Matthew Sathe, B.S. Study completion date – July, 2, 2014. Project Number A16832.**

This study was conducted against Vancomycin Resistant *Enterococcus faecalis* – VRE (ATCC 51575) for product Micro-Kill Bleach Germicidal Bleach Solution (Lot# E27S and Lot# E28S). These were tested using ATS Laboratory Protocol No. MDI01051514.GS.5 (copy provided). The product was received as a ready to use liquid and tested as a trigger spray. A loopful of stock slant culture was transferred to an initial 10 mL tube of growth medium, mixed, and the initial culture was incubated for 24±2 hours at 35-37°C. Following incubation, a 10 µL aliquot of culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1). The final test culture was incubated for 48-54 hours at 35-37°C. The test culture was vortex mixed for 3 to 4 seconds and allowed to stand for ≥10 minutes prior to use. Then the upper portion of the culture was removed, leaving behind any clumps or debris and was pooled in a sterile vessel and mixed. The final test culture was mixed thoroughly prior to use. A 0.10 mL aliquot of FBS was added to 1.90 mL of prepared culture to yield a 5% fetal bovine serum organic soil load. Individual sterile glass slide carriers (18 mm x 36 mm), each in a Petri dish matted with two pieces of filter paper, were inoculated with 10.0 µL of test organism using a calibrated pipettor. Inoculum was uniformly spread over the test surface (approx. 1 square inch) of the slide and covered immediately. This was repeated until all slides were individually inoculated. The culture was vortex mixed periodically during inoculation as necessary. The slides were allowed to dry for 33 minutes at 27.2-27.7°C and at 65% relative humidity, appeared visibly dry, and were used within 2 hours of drying. For each lot of test substance, test carriers (in an

undisturbed horizontal position) were sprayed with the test substance at staggered intervals at a distance of 6-8 inches using 4 sprays, and sprayed within ±3 seconds of the exposure time following a calibrated timer. Each treated carrier was then held at room temperature (21.5°C) and 46.0% relative humidity for 30 seconds. After exposure, excess liquid was drained off the carrier without touching the carrier to the Petri dish or filter paper. Each carrier was then transferred using sterile forceps following identical staggered intervals to 20 mL aliquots of neutralizing subculture medium. The vessel was shaken thoroughly. All subcultures were incubated for 48±2 hours at 35-37°C, and then visually examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation and carrier population.

28. MRID 494865-24, "AOAC Germicidal Spray Method," Test Organism: *Streptococcus pneumoniae* (ATCC 6305) for product Micro-Kill Bleach Germicidal Bleach Solution (Lot # E27S and Lot # E28S). Study conducted at ATS Labs by Matthew Sathe. Study completion date – July 11, 2014. Project Number A16833.

This study was conducted against *Streptococcus pneumoniae* (ATCC 6305) for product Micro-Kill Bleach Germicidal Bleach Solution (Lot # E27S and Lot # E28S) using ATS Laboratory Protocol No. MDI01051514.GS.14 (copy provided). The product was received as a ready to use liquid and tested as a trigger spray. A culture of the test organism was prepared by using a stock plate to inoculate multiple agar plates containing Tryptic Soy Agar with 5% Sheep Blood (BAP) and incubating for 2 days at 35-37°C in CO_2. Following incubation, an organism suspension was prepared in Fluid Thioglycollate Medium to target 1 x 10^8 CFU/mL. A spec value of 1.821 at 620 nm was prepared. The final test culture was mixed thoroughly prior to use. A 0.10 mL aliquot of FBS was added to 1.90 mL of the prepared culture to yield a 5% fetal bovine serum organic soil load. Ten (10) individual sterile glass slide carriers (18 mm x 36 mm) per batch, each in a Petri dish matted with two pieces of filter paper, were inoculated with 10.0 µL of test organism using a calibrated pipettor. Inoculum was uniformly spread over the test surface (approx. 1 square inch) of the slide and covered immediately. This was repeated until all slides were individually inoculated. The culture was vortex mixed periodically during inoculation as necessary. The carriers were allowed to dry for 30 minutes at 25-30°C at 65% relative humidity and appeared visibly dry, and were used in the test procedure within 2 hours of drying. For each lot of test substance, test carriers (in an undisturbed horizontal position) were sprayed with the test substance at staggered intervals at a distance of 6-8 inches using 4 sprays, and sprayed within ±3 seconds of the exposure time following a calibrated timer. Following treatment, each carrier was held at room temperature (20.8°C) and 50.5% relative humidity for 30 seconds. After exposure time, excess liquid was drained off the carrier without touching the carrier to the Petri dish or filter paper. Each carrier was then transferred using sterile forceps and following identical staggered intervals to 20 mL aliquots of primary neutralizing subculture medium. The vessel was shaken thoroughly. The carriers were transferred into individual secondary subcultures containing 20 mL aliquots of secondary subculture medium (same as primary) within approximately 25-60 minutes of the initial transfer and the vessel was shaken thoroughly. All subcultures were incubated for 46-50 hours at 35-37°C in CO_2 the stored. Following incubation and storage, the subcultures were visually examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation and carrier population.

29. MRID 494865-29, "AOAC Germicidal Spray Method," Test Organism: *Legionella pneumophila* (ATCC 33153). For product Micro-Kill Bleach Germicidal Bleach Solution, Lots #E27S and #E28S. Study conducted at ATS Labs by Matthew Sathe. Study completion date – August 12, 2014. Project Number A16834.

This study was conducted against *Legionella pneumophila* (ATCC 33153). Two lots (Lot

Nos. E27S and E28S) of the product, Micro-Kill Bleach Germicidal Bleach Solution, were tested using ATS Laboratory Protocol No. MDI01051514.GS.15 (copy provided). The product was received as a ready to use liquid and tested as a trigger spray. From stock, sufficient plates were inoculated with the test organism and incubated for 2 days at 35-37°C in CO_2. Following incubation, the test organism was suspended in Butterfield's buffer to approximately match a 2.0 McFarland Turbidity Standard. The final test culture was mixed thoroughly prior to use. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to achieve a 5% organic soil load. A 10 µL aliquot of the prepared culture was uniformly spread over 10 individual glass slide carriers (18 mm x 36 mm) per product batch, each in a Petri dish matted with two pieces of filter paper. Inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The carriers were allowed to dry for 30 minutes at 35-37°C (36.2°C) and at a 55.8% relative humidity. Carriers were used within 2 hours of drying. For each lot of test substance, test carriers were sprayed in a horizontal position, at staggered intervals with the test substance at a distance of 6-8 inches until thoroughly wet (4 full sprays). The carriers were allowed to remain wet for 30 seconds at room temperature (21°C) and at 46.9% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at identical staggered intervals to 20 mL neutralize. The vessel was shaken thoroughly, vortex mixed, and the subculture broths transferred to a filter membrane pre-wetted with 10 mL of sterile saline and filtered using a vacuum pump. Each filter membrane was washed with ≥50 mL of sterile saline, removed aseptically from the filter unit and placed on the surface of a BCYE agar plate for recovery of the test organism. All subcultures were incubated for 3 days at 35-37°C in CO_2. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation and carrier population.

30. MRID 494865-30, "AOAC Germicidal Spray Method," Test Organism: Linezolid Resistant *Staphylococcus aureus* (NRS 119) for product Micro-Kill Bleach Germicidal Bleach Solution (Lot# E27S and Lot# E28S). Study conducted at ATS Labs by Matthew Sathe, B.S. Study completion date – July 9, 2014. Project Number A16835.

This study was conducted against Linezolid Resistant *Staphylococcus aureus* (LRS 119) for product Micro-Kill Bleach Germicidal Bleach Solution (Lot# E27S and Lot# E28S). These were tested using ATS Laboratory Protocol No. MDI01051714.GS.8 (copy provided). The product was received as a ready to use liquid and tested as a trigger spray. A loopful of stock slant culture was transferred to an initial 10 mL tube of growth medium, mixed, and the initial culture was incubated for 24±2 hours at 35-37°C. Following incubation, a 10 µL aliquot of culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1). The final test culture was incubated for 48-54 hours at 35-37°C. The test culture was vortex mixed for 3 to 4 seconds and allowed to stand for ≥10 minutes prior to use. Then the upper portion of the culture was removed, leaving behind any clumps or debris and was pooled in a sterile vessel and mixed. The final test culture was mixed thoroughly prior to use. A 0.10 mL aliquot of FBS was added to 1.90 mL of prepared culture to yield a 5% fetal bovine serum organic soil load. The final test culture was mixed thoroughly prior to use. A 0.10 mL aliquot of FBS was added to 1.90 mL of prepared culture to yield a 5% fetal bovine serum organic soil load. Ten (10) individual sterile glass slide carriers (18 mm x 36 mm) per batch, each in a Petri dish matted with two pieces of filter paper, were inoculated with 10.0 µL of test organism using a calibrated pipettor. Inoculum was uniformly spread over the test surface (approx. 1 square inch) of the slide and covered immediately. This was repeated until all slides were individually inoculated. The culture was vortex mixed periodically during inoculation as necessary. The slides were allowed to dry for 30 minutes

at 36.1-36.5°C at 55.4% relative humidity, appeared visibly dry, and were used within 2 hours of drying. For each lot of test substance, test carriers (in an undisturbed horizontal position) were sprayed with the test substance at staggered intervals at a distance of 6-8 inches using 4 sprays, and sprayed within ±3 seconds of the exposure time following a calibrated timer. Each treated carrier was then held at room temperature (21.0°C) and 46.9% relative humidity for 30 seconds. After exposure, excess liquid was drained off the carrier without touching the carrier to the Petri dish or filter paper. Each carrier was then transferred using sterile forceps. The vessel was shaken thoroughly. All subcultures were incubated for 48±2 hours at 35-37°C, stored at 2-8°C for 1 day, and then visually examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation and carrier population.

31. MRID 494865-31, "AOAC Germicidal Spray Method," Test Organism: Vancomycin Intermediate Resistant *Staphylococcus aureus* - VISA (HIP 5836) for product Micro-Kill Bleach Germicidal Bleach Solution (Lot# E27S and Lot# E28S). Study conducted at ATS Labs by Matthew Sathe, B.S. Study completion date – August 5, 2014. Project Number A16836.

This study was conducted against Vancomycin Intermediate Resistant *Staphylococcus aureus* -VISA (HIP 5836) for product Micro-Kill Bleach Germicidal Bleach Solution (Lot# E27S and Lot# E28S). These were tested using ATS Laboratory Protocol No. MDI01051714.GS.12 (copy provided). The product was received as a ready to use liquid and tested as a trigger spray. From stock, multiple agar plates were inoculated and incubated for 2 days at 35-37°C. Following incubation, the organism was suspended in sterile Butterfield's Buffer to match a 0.5 McFarland turbidity standard. The final test culture was mixed thoroughly prior to use. A 0.10 mL aliquot of FBS was added to 1.90 mL of prepared culture to yield a 5% fetal bovine serum organic soil load. Ten (10) individual sterile glass slide carriers (18 mm x 36 mm) per batch, each in a Petri dish matted with two pieces of filter paper, were inoculated with 10.0 µL of test organism using a calibrated pipettor. Inoculum was uniformly spread over the test surface (approx. 1 square inch) of the slide and covered immediately. This was repeated until all slides were individually inoculated. The culture was vortex mixed periodically during inoculation as necessary. The slides were allowed to dry for 31 minutes at 36.0°C at 40% relative humidity, appeared visibly dry, and were used within 2 hours of drying. For each lot of test substance, test carriers (in an undisturbed horizontal position) were sprayed with the test substance at staggered intervals at a distance of 6-8 inches using 4 sprays, and sprayed within ±3 seconds of the exposure time following a calibrated timer. Each treated carrier was then held at room temperature (21.53°C) and 42.96% relative humidity for 1 minute. After exposure, excess liquid was drained off the carrier without touching the carrier to the Petri dish or filter paper. Each carrier was then transferred using sterile forceps following identical staggered intervals to 20 mL aliquots of neutralizing subculture medium. The vessel was shaken thoroughly. All subcultures were incubated for 48±2 hours at 35-37°C, and then visually examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation and carrier population.

32. MRID 494865-32, "AOAC Germicidal Spray Method," Test Organism: *Bordetella pertussis* (ATCC 12743). For product Micro-Kill Bleach Germicidal Bleach Solution, Lots #E27S and #E28S. Study conducted at ATS Labs by Matthew Sathe. Study completion date – July 14, 2014. Project Number A16837.

This study was conducted against *Bordetella pertussis* (ATCC 12743). Two lots (Lot Nos. E27S and E28S) of the product, Micro-Kill Bleach Germicidal Bleach Solution, were tested using ATS Laboratory Protocol No. MDI01051714.GS.13 (copy provided). The product was received as a ready to use liquid and tested as a trigger spray. From stock, sufficient agar plates were

inoculated with the test organism and incubated for 3 days at 35-37°C. Following incubation, the test organism was suspended in Butterfield's buffer to approximately match a 3.0 McFarland Turbidity Standard. The final test culture was mixed thoroughly prior to use. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to achieve a 5% organic soil load. A 10 µL aliquot of the prepared culture was uniformly spread over 10 individual glass slide carriers (18 mm x 36 mm) per product batch, each in a Petri dish matted with two pieces of filter paper. Inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The carriers were allowed to dry for 30 minutes at 35-37°C (36.1-36.2°C) and at a 40% relative humidity. Carriers were used within 2 hours of drying. For each lot of test substance, test carriers were sprayed in a horizontal position, at staggered intervals with the test substance at a distance of 6-8 inches until thoroughly wet (4 sprays). The carriers were allowed to remain wet for 30 seconds at room temperature (21.0°C) and at 47.1% relative humidity. The subcultures were then vortex mixed. The entire volume of the subculture broths were individually transferred to the surface of a filter membrane pre-wetted with saline and filtered using a vacuum pump and washed with saline. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at identical staggered intervals to 20 mL of neutralizer medium. The vessel was shaken thoroughly. Within 30 minutes of neutralization, the entire volume of the subculture broths were individually transferred to the surface of a filter membrane (0.2 µm porosity) pre-wetted with 10.0 mL of sterile saline and filtered using a vacuum pump. Each filter membrane was washed with ≥50 mL of sterile saline, removed aseptically from the filter unit, and placed on the surface of a Bordet Gengou Agar plate for recovery of the test organism. All subcultures were incubated for 5 days at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation and carrier population.

33. MRID 494865-33, "AOAC Germicidal Spray Method," Test Organism: *Campylobacter jejuni* (ATCC 29428). For product Micro-Kill Bleach Germicidal Bleach Solution, Lots #E27S and #E28S. Study conducted at ATS Labs by Matthew Sathe. Study completion date – July 11, 2014. Project Number A16838.

This study was conducted against *Campylobacter jejuni* (ATCC 29428). Lot Nos. E27S and E28S of the product, Micro-Kill Bleach Germicidal Bleach Solution, were tested using ATS Laboratory Protocol No. MDI01051714.GS.14 (copy provided). The product was received as a ready to use liquid and tested as a trigger spray. A stock plate of the test organism was created on Tryptic Soy Agar with 5% Sheep Blood (BAP) and incubated for 3 days at 35-37°C under microaerophilic conditions in a CampyPak™ Plus pack. From there, multiple BAP were inoculated and incubated for 2 days at 35-37°C in a CampyPak™ Plus pack. Finally, a bacterial suspension was prepared by swabbing bacterial growth and placing the swab in Fluid Thioglycollate Medium to target approximately $1.0x10^8$ CFU/mL and mixed prior to use. A 0.10 mL aliquot of FBS was added to 1.90 mL of broth culture to yield a 5% fetal bovine serum organic soil load. Ten individual glass slide carriers (18 mm x 36 mm) per product batch, each in a Petri dish matted with two pieces of filter, were inoculated with 10.0 µL of test organism using a calibrated pipettor. Inoculum was uniformly spread over the test surface of the slide (approximately 1 square inch) in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 32 minutes at 25-30°C (27.0°C) and at 63% relative humidity. Carriers were used within 2 hours of drying. Test carriers were sprayed in a horizontal position with the test substance at staggered intervals at a distance of 6-8 inches from the carrier surface (4 full sprays). The carriers were allowed to remain wet for 30 seconds at room temperature 18-25°C (21.4°C) and at 40.8% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile

forceps at identical staggered intervals to 20 mL of neutralizer broth. The vessel was shaken thoroughly and vortex mixed for 120±5 seconds. The entire volume of the subculture broths were individually transferred to the surface of a filter membrane (0.45 µm porosity) pre-wetted with 10.0 mL of sterile saline and filtered using a vacuum pump. Each filter membrane was washed with ≥50 mL of sterile saline, then was removed aseptically from the filter unit and placed on the surface of a BAP plate and incubated for 2 days at 35-37°C under microaerophilic conditions in a CampyPak™ Plus pack. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population.

34. MRID 494865-34, "Fungicidal Germicidal Spray Method," Test Organism: *Aspergillus brasiliensis* (ATCC 16404), for product Micro-Kill Bleach Germicidal Bleach Solution, Lot# E27S and Lot# E28S. Study conducted at ATS Labs by Kristen Niehaus. Study completion date – July 10, 2014. Project Number A16839.

This study was conducted against *Aspergillus brasiliensis* (ATCC 16404) for product Micro-Kill Bleach Germicidal Bleach Solution, Lot# E27S and Lot# E28S. These were tested using ATS Laboratory Protocol No. MDI01051714.FGS.2 (copy provided). The product was received as a ready to use liquid and tested as a trigger spray. From a stock culture slant of the test organism, a loopful of culture was transferred to a sufficient number of 10 mL tubes of Sabouraud Agar modified and incubated for 10 days at 25-30°C. Following incubation, saline/Triton Solution and sterile glass beads were added to the flask. The flask was agitated to remove mycelia/conidia from the agar. The conidial concentration was estimated by counting in a hemacytometer. The conidial count was determined to be $1.1x10^8$ conidia/mL. The final test culture was mixed thoroughly prior to use. A 0.10 mL aliquot of FBS was added to 1.90 mL of prepared culture to yield a 5% fetal bovine serum organic soil load. Ten individual sterile glass slide carriers per lot (18 mm x 36 mm), each in a Petri dish matted with two pieces of filter paper, were inoculated with 10.0 µL of test organism using a calibrated pipettor. Inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 31 minutes at 35-37°C (36.1-36.2°C) and at 56.8% relative humidity. Carriers were used within 2 hours of drying. For each lot of test substance, test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface using 4 full sprays. The carriers were allowed to remain wet for 1 minute at room temperature (24.3°C) and at 48.2% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at identical staggered intervals to 20 mL of subculture medium, Sabouraud Dextrose Broth + 0.07% Lecithin + 0.5% Tween 80, and 0.05% sodium thiosulfate to neutralize. All neutralized subcultures were incubated for 10 days at 25-30°C. Agar plate subcultures were incubated at 25-30°C for 44-76 hours. Agar plate subcultures were stored at 2-8°C for 2 days prior to examination. Following incubation and storage, the subcultures were examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation and carrier population

35. MRID 494865-35. "AOAC Germicidal Spray Method," Test Organism: Extended-Spectrum beta-lactamase (ESBL) positive *Escherichia coli* (ATCC BAA-196) for product Micro-Kill Bleach Germicidal Bleach Solution (Lot # E27S and Lot # E28S). Study conducted at ATS Labs by Jill Ruhme, B.S. Study completion date – July 9, 2014. Project Number A16840.

This study was conducted against Extended-Spectrum beta-lactamase (ESBL) positive *Escherichia coli* (ATCC BAA-196) for product Micro-Kill Bleach Germicidal Bleach Solution (Lot #

E27S and Lot # E28S) using ATS Laboratory Protocol No. MDI01051514.GS.3 (copy provided). The product was received as a ready to use liquid and tested as a trigger spray. A loopful of stock slant culture was transferred to an initial 10 mL tube of growth medium (Synthetic Broth). The tube was mixed and the initial culture was incubated for 24±2 hours at 35-37°C. Following incubation a 10 µL aliquot of culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1). The final test culture was incubated for 48-54 hours at 35-37°C. The test culture was vortex mixed for 3 to 4 seconds and allowed to stand for ≥10 minutes prior to use. After this time, the upper portion of the culture was removed, leaving behind any clumps or debris and was pooled in a sterile vessel and mixed. The final test culture was mixed thoroughly prior to use. A 0.10 mL aliquot of FBS was added to 1.90 mL of prepared culture to yield a 5% fetal bovine serum organic soil load. Ten (10) individual sterile glass slide carriers (18 mm x 36 mm) per batch, each in a Petri dish matted with two pieces of filter paper, were inoculated with 10.0 µL of test organism using a calibrated pipettor. Inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The culture was vortex mixed periodically during inoculation as necessary. The carriers were allowed to dry for 32 minutes at 36.0°C at 40% relative humidity and appeared visibly dry, and were used in the test procedure within 2 hours of drying. For each lot of test substance, test carriers (in an undisturbed horizontal position) were sprayed with the test substance at staggered intervals at a distance of 6-8 inches using 4 sprays. The carrier was sprayed with the test substance within ±3 seconds of the exposure time following a calibrated timer. Following the spray treatment, each treated carrier was held at room temperature (21.8°C) and 48.8% relative humidity for 30 seconds. At the end of the exposure time, the excess liquid was drained off the carrier without touching the carrier to the Petri dish or filter paper. Each treated carrier was then transferred using sterile forceps and following identical staggered intervals to 20 mL aliquots of neutralizing subculture medium (Letheen Broth + 1.0% sodium thiosulfate). The vessel was shaken thoroughly. All subcultures were incubated for 48±2 hours at 35-37°C, then stored at 2-8°C for 2 days, then visually examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation and carrier population.

36. MRID 494865-36, "AOAC Germicidal Spray Method," Test Organism: *Escherichia coli* (ATCC 11229). For product Micro-Kill Bleach Germicidal Bleach Solution, Lots #E27S and #E28S. Study conducted at ATS Labs by Kristen Niehaus. Study completion date – July 3, 2014. Project Number A16850.

This study was conducted against *Escherichia coli* (ATCC 11229). Two lots (Lot Nos. E27S and E28S) of the product, Micro-Kill Bleach Germicidal Bleach Solution, were tested using ATS Laboratory Protocol No. MDI01051514.GS.9 (copy provided). The product was received as a ready to use liquid and tested as a trigger spray. A loopful of a stock slant culture was transferred to an initial 10 mL tube of growth medium, mixed and incubated for 24±2 hours at 35-37°C. A 10 µL aliquot of culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (1st daily transfer). The final test culture was incubated for 48-54 hours at 35-37°C. The test culture was vortex mixed (3-4 seconds) and allowed to stand ≥10 minutes prior to use. The upper portion was removed and pooled in a sterile vessel and mixed. The culture was diluted by combining 2.0 mL of test organism suspension with 2.0 mL of sterile growth medium. The final test culture was mixed thoroughly prior to use. A 0.1 mL aliquot of fetal bovine serum (FBS) was added to 1.9 mL of prepared culture to achieve a 5% organic soil load. A 10 µL aliquot of the prepared culture was uniformly spread over 10 individual glass slide carriers (18 mm x 36 mm) per product batch, each in a Petri dish matted with two pieces of filter paper. Inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish

and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 32 minutes at 35-37°C (36.0-36.2°C) and at 54.4% relative humidity. Carriers were used within 2 hours of drying. Test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface until thoroughly wet (4 full sprays). The carriers were allowed to remain wet for 30 seconds at room temperature room temperature (21.5°C) and 46.5% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at identical staggered intervals to 20 mL of neutralizer broth and shaken thoroughly. All subcultures were incubated for 48±2 hours at 35-37°C. Subcultures were stored at 2-8°C for 2 days prior to examination. Following incubation and storage, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

37. MRID 494865-37, "AOAC Germicidal Spray Method," Test Organism: Extended-Spectrum beta-lactamase (ESBL) positive *Klebsiella pneumoniae* (ATCC 700603) for product Micro-Kill Bleach Germicidal Bleach Solution (Lot# E27S and Lot# E28S). Study conducted at ATS Labs by Jill Ruhme. Study completion date – July 10, 2014. Project Number A16841.

This study was conducted against Extended-Spectrum beta-lactamase (ESBL) positive *Klebsiella pneumoniae* (ATCC 700603) for product Micro-Kill Bleach Germicidal Bleach Solution (Lot# E27S and Lot# E28S) using ATS Laboratory Protocol No. MDI01051514.GS.4 (copy provided). The product was received as a ready to use liquid and tested as a trigger spray. A loopful of stock slant culture was transferred to an initial 10 mL tube of growth medium (Nutrient Broth). The tube was mixed and the initial culture was incubated for 24±2 hours at 35-37°C. Following incubation a 10 µL aliquot of culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1). The final test culture was incubated for 48-54 hours at 35-37°C. The test culture was vortex mixed for 3 to 4 seconds and allowed to stand for ≥10 minutes prior to use. After this time, the upper portion of the culture was removed, leaving behind any clumps or debris and was pooled in a sterile vessel and mixed. The culture was diluted by combining 2.0 mL of test organism suspension with 2.0 mL of sterile growth medium. The final test culture was mixed thoroughly prior to use. A 0.10 mL aliquot of FBS was added to 1.90 mL of prepared culture to yield a 5% fetal bovine serum organic soil load. Ten (10) individual sterile glass slide carriers (18 mm x 36 mm) per batch, each in a Petri dish matted with two pieces of filter paper, were inoculated with 10.0 µL of test organism using a calibrated pipettor. Inoculum was uniformly spread over the test surface (approx. 1 square inch) of the slide and covered immediately. This was repeated until all slides were individually inoculated. The culture was vortex mixed periodically during inoculation as necessary. The carriers were allowed to dry for 30 minutes at 35-37°C at 53.8% relative humidity and appeared visibly dry, and were used in the test procedure within 2 hours of drying. For each lot of test substance, test carriers (in an undisturbed horizontal position) were sprayed with the test substance at staggered intervals at a distance of 6-8 inches using 4 sprays, and sprayed within ±3 seconds of the exposure time following a calibrated timer. Following treatment, each carrier was held at room temperature (20.8°C) and 47.7% relative humidity for 30 seconds. After exposure time, excess liquid was drained off the carrier without touching the carrier to the Petri dish or filter paper. Each carrier was then transferred using sterile forceps and following identical staggered intervals to 20 mL aliquots of neutralizing subculture medium. The vessel was shaken thoroughly. All subcultures were incubated for 48±2 hours at 35-37°C and then visually examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation and carrier population.

38. MRID 494865-38, "AOAC Germicidal Spray Method," Test Organism: Multi-Drug Resistant (MDR) *Enterococcus faecium* (ATCC 51559) for product Micro-Kill Bleach Germicidal Bleach Solution (Lot# E27S and Lot# E28S). Study conducted at ATS Labs by Jill Ruhme. Study completion date – November 11, 2014. Project Number A16842.

This study was conducted against Multi-Drug Resistant (MDR) *Enterococcus faecium* (ATCC 51559) for product Micro-Kill Bleach Germicidal Bleach Solution (Lot# E27S and Lot# E28S) using ATS Laboratory Protocol No. MDI01051514.GS.7 (copy provided). The product was received as a ready to use liquid and tested as a trigger spray. A loopful of stock slant culture was transferred to an initial 10 mL tube of growth medium. The tube was mixed and the initial culture was incubated for 24±2 hours at 35-37°C. Following incubation a 10 µL aliquot of culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1). The final test culture was incubated for 48-54 hours at 35-37°C. The test culture was vortex mixed for 3 to 4 seconds and allowed to stand for ≥10 minutes prior to use. After this time, the upper portion of the culture was removed, leaving behind any clumps or debris and was pooled in a sterile vessel and mixed. The final test culture was mixed thoroughly prior to use. A 0.10 mL aliquot of FBS was added to 1.90 mL of prepared culture to yield a 5% fetal bovine serum organic soil load. Ten (10) individual sterile glass slide carriers (18 mm x 36 mm) per batch, each in a Petri dish matted with two pieces of filter paper, were inoculated with 10.0 µL of test organism using a calibrated pipettor. Inoculum was uniformly spread over the test surface (approx. 1 square inch) of the slide and covered immediately. This was repeated until all slides were individually inoculated. The culture was vortex mixed periodically during inoculation as necessary. The carriers were allowed to dry for 31 minutes at 27.0°C at 65% relative humidity and appeared visibly dry, and were used in the test procedure within 2 hours of drying. For each lot of test substance, test carriers (in an undisturbed horizontal position) were sprayed with the test substance at staggered intervals at a distance of 6-8 inches using 4 sprays, and sprayed within ±3 seconds of the exposure time following a calibrated timer. Following treatment, each carrier was held at room temperature (21.2°C) and 52.9% relative humidity for 30 seconds. After exposure time, excess liquid was drained off the carrier without touching the carrier to the Petri dish or filter paper. Each carrier was then transferred using sterile forceps and following identical staggered intervals to 20 mL aliquots of neutralizing subculture medium. The vessel was shaken thoroughly. All subcultures were incubated for 48±2 hours at 35-37°C and then visually examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation and carrier population.

39. MRID 494865-39, "AOAC Germicidal Spray Method," Test Organism: Community Acquired Methicillin Resistant *Staphylococcus aureus* -CA-MRSA Genotype USA 300 (NRS 384) for product Micro-Kill Bleach Germicidal Bleach Solution (Lot# E27S and Lot# E28S). Study conducted at ATS Labs by Jill Ruhme. Study completion date – July 9, 2014. Project Number A16843.

This study was conducted against Community Acquired Methicillin Resistant *Staphylococcus aureus* -CA-MRSA Genotype USA 400 (NRS 384) for product Micro-Kill Bleach Germicidal Bleach Solution (Lot# E27S and Lot# E28S). These were tested using ATS Laboratory Protocol No. MDI01051714.GS.3 (copy provided). The product was received as a ready to use liquid and tested as a trigger spray. A loopful of stock slant culture was transferred to an initial 10 mL tube of Synthetic Broth growth medium, mixed, and the initial culture was incubated for 24±2 hours at 35-37°C. Following incubation, a 10 µL aliquot of culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1). The final test culture was incubated for 48-54 hours at 35-37°C. The test culture was vortex mixed for 3 to 4

seconds and allowed to stand for ≥10 minutes prior to use. Then the upper portion of the culture was removed, leaving behind any clumps or debris and was pooled in a sterile vessel and mixed. The final test culture was mixed thoroughly prior to use. A 0.10 mL aliquot of FBS was added to 1.90 mL of prepared culture to yield a 5% fetal bovine serum organic soil load. Ten (10) individual sterile glass slide carriers (18 mm x 36 mm) per batch, each in a Petri dish matted with two pieces of filter paper, were inoculated with 10.0 µL of test organism using a calibrated pipettor. Inoculum was uniformly spread over the test surface (approx. 1 square inch) of the slide and covered immediately. This was repeated until all slides were individually inoculated. The culture was vortex mixed periodically during inoculation as necessary. The slides were allowed to dry for 30 minutes at 36.1-36.2°C and at 51.9% relative humidity, appeared visibly dry, and were used within 2 hours of drying. For each lot of test substance, test carriers (in an undisturbed horizontal position) were sprayed with the test substance at staggered intervals at a distance of 6-8 inches using 4 sprays, and sprayed within ±3 seconds of the exposure time following a calibrated timer. Each treated carrier was then held at room temperature (21.5°C) and 44.20% relative humidity for 30 seconds. After exposure, excess liquid was drained off the carrier without touching the carrier to the Petri dish or filter paper. Each carrier was then transferred using sterile forceps following identical staggered intervals to 20 mL aliquots of neutralizing subculture medium (Letheen Broth + 1.0% sodium thiosulfate). The vessel was shaken thoroughly. All subcultures were incubated for 48±2 hours at 35-37°C and then visually examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation and carrier population.

40. MRID 494865-40, "AOAC Germicidal Spray Method," Test Organism: Community Acquired Methicillin Resistant *Staphylococcus aureus* -CA-MRSA Genotype USA 400 (NRS 123) for product Micro-Kill Bleach Germicidal Bleach Solution (Lot# E27S and Lot# E28S). Study conducted at ATS Labs by Jill Ruhme. Study completion date – July 9, 2014. Project Number A16844.

This study was conducted against Community Acquired Methicillin Resistant *Staphylococcus aureus* -CA-MRSA Genotype USA 400 (NRS 123) for product Micro-Kill Bleach Germicidal Bleach Solution (Lot# E27S and Lot# E28S). These were tested using ATS Laboratory Protocol No. MDI01051714.GS.4 (copy provided). The product was received as a ready to use liquid and tested as a trigger spray. A loopful of stock slant culture was transferred to an initial 10 mL tube of Synthetic Broth growth medium, mixed, and the initial culture was incubated for 24±2 hours at 35-37°C. Following incubation, a 10 µL aliquot of culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1). The final test culture was incubated for 48-54 hours at 35-37°C. The test culture was vortex mixed for 3 to 4 seconds and allowed to stand for ≥10 minutes prior to use. Then the upper portion of the culture was removed, leaving behind any clumps or debris and was pooled in a sterile vessel and mixed. The final test culture was mixed thoroughly prior to use. A 0.10 mL aliquot of FBS was added to 1.90 mL of prepared culture to yield a 5% fetal bovine serum organic soil load. Ten (10) individual sterile glass slide carriers (18 mm x 36 mm) per batch, each in a Petri dish matted with two pieces of filter paper, were inoculated with 10.0 µL of test organism using a calibrated pipettor. Inoculum was uniformly spread over the test surface (approx. 1 square inch) of the slide and covered immediately. This was repeated until all slides were individually inoculated. The culture was vortex mixed periodically during inoculation as necessary. The slides were allowed to dry for 32 minutes at 35.9-36.1°C and at 51.9% relative humidity, appeared visibly dry, and were used within 2 hours of drying. For each lot of test substance, test carriers (in an undisturbed horizontal position) were sprayed with the test substance at staggered intervals at a distance of 6-8 inches using 4 sprays, and sprayed within ±3 seconds of the exposure time following a calibrated timer. Each treated carrier was then held at room temperature (21.6°C) and 45.7% relative humidity for 30 seconds.

After exposure, excess liquid was drained off the carrier without touching the carrier to the Petri dish or filter paper. Each carrier was then transferred using sterile forceps following identical staggered intervals to 20 mL aliquots of neutralizing subculture medium (Letheen Broth + 1.0% sodium thiosulfate). The vessel was shaken thoroughly. All subcultures were incubated for 48±2 hours at 35-37°C and then visually examined for the presence or absence of visible growth. One of the 10 test carriers exhibited growth of the target organism; so this study was repeated using 60 test carriers. Controls included those for purity, sterility, viability, neutralization confirmation and carrier population.

41. MRID 494865-41, "AOAC Germicidal Spray Method," Test Organism: *Escherichia coli* O157:H7 (ATCC 35150). For product Micro-Kill Bleach Germicidal Bleach Solution, Lots #E27S and #E28S. Study conducted at ATS Labs by Jill Ruhme. Study completion date – July 8, 2014. Project Number A16845.

This study was conducted against *Escherichia coli* O157:H7 (ATCC 35150). Two lots (Lot Nos. E27S and #E28S) of the product, Micro-Kill Bleach Germicidal Bleach Solution, were tested using ATS Laboratory Protocol No. MDI01051714.GS.1 (copy provided). The product was received as a ready to use liquid and tested as a trigger spray. A loop of stock slant culture was transferred to an initial 10 mL tube of growth medium. The tube was mixed and the initial culture was incubated for 24±2 hours at 35-37°C. Following incubation a 10 µL aliquot of culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1). The final test culture was incubated for 48-54 hours at 35-37°C. The test culture was vortex mixed (3-4 seconds) and allowed to stand ≥10 minutes prior to use. The upper portion was removed and pooled in a sterile vessel and mixed. The final test culture was mixed thoroughly prior to use. A 0.1 mL aliquot of fetal bovine serum was added to 1.9 mL of prepared culture to achieve a 5% organic soil load. A 10 µL aliquot of the prepared culture was uniformly spread over 10 individual glass slide carriers (18 mm x 36 mm) per product batch, each in a Petri dish matted with two pieces of filter paper. Inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 31 minutes at 35-37°C and at 53.8% relative humidity. Carriers were used within 2 hours of drying. Test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface until thoroughly wet (4 sprays). The carriers were allowed to remain wet for 30 seconds at room temperature (21.8°C) and at 45.4% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at identical staggered intervals to 20 mL of Letheen Broth + 1.0% sodium thiosulfate to neutralize. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

42. MRID 494865-42, "AOAC Germicidal Spray Method," Test Organism: Vancomycin Resistant *Staphylococcus aureus* -VRSA (VRS1) for product Micro-Kill Bleach Germicidal Bleach Solution (Lot# E27S and Lot# E28S). Study conducted at ATS Labs by Jill Ruhme. Study completion date – June 25, 2014. Project Number A16846.

This study was conducted against Vancomycin Resistant *Staphylococcus aureus* -VRSA (VRS1) for product Micro-Kill Bleach Germicidal Bleach Solution (Lot# E27S and Lot# E28S). These were tested using ATS Laboratory Protocol No. MDI01051714.GS.2 (copy provided). The product was received as a ready to use liquid and tested as a trigger spray. From stock, multiple

agar plates containing Tryptic Soy Agar with 5% Sheep Blood (BAP) were inoculated and incubated for 2 days at 35-37°C. Following incubation, the organism was suspended in sterile Butterfield's Buffer to match a 0.5 McFarland turbidity standard. The final test culture was mixed thoroughly prior to use. A 0.10 mL aliquot of FBS was added to 1.90 mL of prepared culture to yield a 5% fetal bovine serum organic soil load. Ten (10) individual sterile glass slide carriers (18 mm x 36 mm) per batch, each in a Petri dish matted with two pieces of filter paper, were inoculated with 10.0 µL of test organism using a calibrated pipettor. Inoculum was uniformly spread over the test surface (approx. 1 square inch) of the slide and covered immediately. This was repeated until all slides were individually inoculated. The culture was vortex mixed periodically during inoculation as necessary. The slides were allowed to dry for 33 minutes at 35-37°C at 53.2% relative humidity, appeared visibly dry, and were used within 2 hours of drying. For each lot of test substance, test carriers (in an undisturbed horizontal position) were sprayed with the test substance at staggered intervals at a distance of 6-8 inches using 4 sprays, and sprayed within ±3 seconds of the exposure time following a calibrated timer. Each treated carrier was then held at room temperature (20.9°C) and 50.4% relative humidity for 30 seconds. After exposure, excess liquid was drained off the carrier without touching the carrier to the Petri dish or filter paper. Each carrier was then transferred using sterile forceps following identical staggered intervals to 20 mL aliquots of neutralizing subculture medium (Letheen Broth + 1.0% sodium thiosulfate). The vessel was shaken thoroughly. All subcultures were incubated for 48±2 hours at 35-37°C, stored at 2-8°C for 2 days, and then visually examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation and carrier population.

43. MRID 494865-43, "AOAC Germicidal Spray Method," Test Organism: Carbapenem Resistant *Klebsiella pneumoniae* (ATCC BAA-1705) for product Micro-Kill Bleach Germicidal Bleach Solution (Lot # E27S and Lot # E28S). Study conducted at ATS Labs by Jill Ruhme, B.S. Study completion date – June 27, 2014. Project Number A16847.

This study was conducted against Carbapenem Resistant *Klebsiella pneumoniae* (ATCC BAA-1705) for product Micro-Kill Bleach Germicidal Bleach Solution (Lot # E27S and Lot # E28S) using ATS Laboratory Protocol No. MDI01051514.GS.12 (copy provided). The product was received as a ready to use liquid and tested as a trigger spray. A loopful of stock slant culture was transferred to an initial 10 mL tube of growth medium (Nutrient Broth). The tube was mixed and the initial culture was incubated for 24±2 hours at 35-37°C. Following incubation a 10 µL aliquot of culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1). The final test culture was incubated for 48-54 hours at 35-37°C. The test culture was vortex mixed for 3 to 4 seconds and allowed to stand for ≥10 minutes prior to use. After this time, the upper portion of the culture was removed, leaving behind any clumps or debris and was pooled in a sterile vessel and mixed. The culture was diluted using sterile growth medium by combining 2.00 mL of test organism suspension with 2.00 mL of sterile growth medium The final test culture was mixed thoroughly prior to use. A 0.10 mL aliquot of FBS was added to 1.90 mL of prepared culture to yield a 5% fetal bovine serum organic soil load. Ten (10) individual sterile glass slide carriers (18 mm x 36 mm) per batch, each in a Petri dish matted with two pieces of filter paper, were inoculated with 10.0 µL of test organism using a calibrated pipettor. Inoculum was uniformly spread over the test surface (approx. 1 square inch) of the slide and covered immediately. This was repeated until all slides were individually inoculated. The culture was vortex mixed periodically during inoculation as necessary. The carriers were allowed to dry for 30 minutes at 35-37°C at 40% relative humidity and appeared visibly dry, and were used in the test procedure within 2 hours of drying. For each lot of test substance, test carriers (in an undisturbed horizontal position) were sprayed with the test substance at staggered intervals at a distance of 6-8 inches using 4 sprays, and sprayed within ±3 seconds of the exposure time

following a calibrated timer. Following treatment, each carrier was held at room temperature (21.2°C) and 48.8% relative humidity for 30 seconds. After exposure time, excess liquid was drained off the carrier without touching the carrier to the Petri dish or filter paper. Each carrier was then transferred using sterile forceps and following identical staggered intervals to 20 mL aliquots of neutralizing subculture medium (Letheen Broth + 1.0% sodium thiosulfate). The vessel was shaken thoroughly. All subcultures were incubated for 48±2 hours at 35-37°C, and then visually examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation and carrier population.

44. MRID 494865-44, "AOAC Germicidal Spray Method," Test Organism: New Delhi metallo-beta-lactamase 1 (NDM-1) positive *Escherichia coli* (CDC 1001728) for product Micro-Kill Bleach Germicidal Bleach Solution (Lot# E27S and Lot# E28S). Study conducted at ATS Labs by Jill Ruhme, B.S. Study completion date – August 11, 2014. Project Number A16848.

This study was conducted against New Delhi metallo-beta-lactamase 1 (NDM-1) positive *Escherichia coli* (CDC 1001728) for product Micro-Kill Bleach Germicidal Bleach Solution (Lot# E27S and Lot# E28S). These were tested using ATS Laboratory Protocol No. MDI01051514.GS.13 (copy provided). The product was received as a ready to use liquid and tested as a trigger spray. A loopful of stock slant culture was transferred to an initial 10 mL tube of Nutrient Broth growth medium, mixed, and the initial culture was incubated for 24±2 hours at 35-37°C. Following incubation, a 10 µL aliquot of culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1). The final test culture was incubated for 48-54 hours at 35-37°C. The test culture was vortex mixed for 3 to 4 seconds and allowed to stand for ≥10 minutes prior to use. Then the upper portion of the culture was removed, leaving behind any clumps or debris and was pooled in a sterile vessel and mixed. The final test culture was mixed thoroughly prior to use. A 0.10 mL aliquot of FBS was added to 1.90 mL of prepared culture to yield a 5% fetal bovine serum organic soil load. Ten (10) individual sterile glass slide carriers (18 mm x 36 mm) per batch, each in a Petri dish matted with two pieces of filter paper, were inoculated with 10.0 µL of test organism using a calibrated pipettor. Inoculum was uniformly spread over the test surface (approx. 1 square inch) of the slide and covered immediately. This was repeated until all slides were individually inoculated. The culture was vortex mixed periodically during inoculation as necessary. The slides were allowed to dry for 30 minutes at 36.0°C and at 40% relative humidity, appeared visibly dry, and were used within 2 hours of drying. For each lot of test substance, test carriers (in an undisturbed horizontal position) were sprayed with the test substance at staggered intervals at a distance of 6-8 inches using 4 sprays, and sprayed within ±3 seconds of the exposure time following a calibrated timer. Each treated carrier was then held at room temperature (21.2°C) and 45.9% relative humidity for 30 seconds. After exposure, excess liquid was drained off the carrier without touching the carrier to the Petri dish or filter paper. Each carrier was then transferred using sterile forceps following identical staggered intervals to 20 mL aliquots of neutralizing subculture medium (Letheen Broth + 0.1% sodium thiosulfate). The vessel was shaken thoroughly. All subcultures were incubated for 48±2 hours at 35-37°C and then visually examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation and carrier population.

45. MRID 494865-45, "AOAC Germicidal Spray Method," Test Organism: *Streptococcus pyogenes* (ATCC 19615). For product Micro-Kill Bleach Germicidal Bleach Solution, Lots #E27S and #E28S. Study conducted at ATS Labs by Kristen Niehaus. Study completion date – June 30, 2014. Project Number A16849.

This study was conducted against *Streptococcus pyogenes* (ATCC 19615). Lot Nos. E27S and E28S of the product, Micro-Kill Bleach Germicidal Bleach Solution, were tested using ATS Laboratory Protocol No. MDI01051514.GS.10 (copy provided). The product was received as a ready to use liquid and tested as a trigger spray. A culture of the test organism was prepared by using a stock plate to inoculate multiple agar plates and incubated for 2 days at 35-37°C in CO_2. An organism suspension was prepared in Fluid Thioglycollate Medium to target approximately $1.0x10^8$ CFU/mL. The final test culture was mixed thoroughly prior to use. A 0.10 mL aliquot of FBS was added to 1.90 mL of broth culture to yield a 5% fetal bovine serum organic soil load. Ten individual glass slide carriers (18 mm x 36 mm) per product batch, each in a Petri dish matted with two pieces of filter, were inoculated with 10.0 µL of test organism using a calibrated pipettor. Inoculum was uniformly spread over the test surface of the slide (approximately 1 square inch) in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 25-30°C (27°C) and at 65% relative humidity. Carriers were used within 2 hours of drying. Test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface until thoroughly wet (4 full sprays). The carriers were allowed to remain wet for 30 seconds at room temperature (21.1°C) and at 58.7% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at identical staggered intervals to 20 mL of Brain Heart Infusion to neutralize. The carriers were transferred into individual secondary subcultures containing 20 mL aliquots of secondary subculture medium within ~25-60 minutes of the initial transfer and the vessel was shaken thoroughly. All subcultures were incubated for 48±2 hours at 35-37°C in CO_2. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population.

46. MRID 494865-46, "AOAC Germicidal Spray Method," Test Organism: *Klebsiella pneumoniae* (ATCC 4352). For product Micro-Kill Bleach Germicidal Bleach Solution, Lots #E27S and #E28S. Study conducted at ATS Labs by Kristen Niehaus. Study completion date – June 30, 2014. Project Number A16851.

This study was conducted against *Klebsiella pneumoniae* (ATCC 4352). Two lots (Lot Nos. E27S and #E28S) of the product, Micro-Kill Bleach Germicidal Bleach Solution, were tested using ATS Laboratory Protocol No. MDI01051514.GS.8 (copy provided). The product was received as a ready to use liquid and tested as a trigger spray. A loop of stock slant culture was transferred to an initial 10 mL tube of growth medium. The tube was mixed and the initial culture was incubated for 24±2 hours at 35-37°C. Following incubation a 10 µL aliquot of culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1). The final test culture was incubated for 48-54 hours at 35-37°C. The test culture was vortex mixed (3-4 seconds) and allowed to stand ≥10 minutes prior to use. The upper portion was removed and pooled in a sterile vessel and mixed. The final test culture was mixed thoroughly prior to use. A 0.1 mL aliquot of fetal bovine serum was added to 1.9 mL of prepared culture to achieve a 5% organic soil load. A 10 µL aliquot of the prepared culture was uniformly spread over 10 individual glass slide carriers (18 mm x 36 mm) per product batch, each in a Petri dish matted with two pieces of filter paper. Inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 33 minutes at 35-37°C (36.1-36.2°C) and at 42% relative humidity. Carriers were used within 2 hours of drying. Test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface until thoroughly wet (4 full sprays). The carriers were allowed to remain wet for 30 seconds at room temperature (21.4°C) and at 46.3% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers

were transferred using sterile forceps at identical staggered intervals to 20 mL of Letheen Broth + 0.1% sodium thiosulfate to neutralize. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

47. MRID 494865-47, "AOAC Germicidal Spray Method," Test Organism: *Enterobacter aerogenes* (ATCC 13048). For product Micro-Kill Bleach Germicidal Bleach Solution, Lots #E27S and #E28S. Study conducted at ATS Labs by Mary J. Miller. Study completion date – July 1, 2014. Project Number A16852.

This study was conducted against *Enterobacter aerogenes* (ATCC 13048). Two lots (Lot Nos. E27S and #E28S) of the product, Micro-Kill Bleach Germicidal Bleach Solution, were tested using ATS Laboratory Protocol No. MDI01051714.GS.5 (copy provided). The product was received as a ready to use liquid and tested as a trigger spray. A loop of stock slant culture was transferred to an initial 10 mL tube of growth medium. The tube was mixed and the initial culture was incubated for 24±2 hours at 25-30°C. Following incubation a 10 µL aliquot of culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1). The final test culture was incubated for 48-54 hours at 25-30°C. The test culture was vortex mixed (3-4 seconds) and allowed to stand ≥10 minutes prior to use. The upper portion was removed and pooled in a sterile vessel and mixed. The culture was diluted by combining 1.0 mL of test organism suspension with 2.0 mL of sterile growth medium. The final test culture was mixed thoroughly prior to use. A 0.1 mL aliquot of fetal bovine serum was added to 1.9 mL of prepared culture to achieve a 5% organic soil load. A 10 µL aliquot of the prepared culture was uniformly spread over the individual glass slide carriers (18 mm x 36 mm) per product batch, each in a Petri dish matted with two pieces of filter paper. Inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 35-37°C (36.1°C) and at 52.1% relative humidity. Carriers were used within 2 hours of drying. Test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface until thoroughly wet (4 full sprays). The carriers were allowed to remain wet for 30 seconds at room temperature (21.34°C) and at 20.84% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at identical staggered intervals to 20 mL of Letheen Broth + 0.1% sodium thiosulfate to neutralize. All subcultures were incubated for 48±2 hours at 25-30°C. Subcultures were stored at 2-8°C for 2 days prior to examination. Following incubation and storage, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

48. MRID 494865-48, "AOAC Germicidal Spray Method," Test Organism: Methicillin Resistant *Staphylococcus aureus* MRSA (ATCC 33592) for product Micro-Kill Bleach Germicidal Bleach Solution (Lot# E27S and Lot# E28S). Study conducted at ATS Labs by Mary J. Miller. Study completion date – July 9, 2014. Project Number A16853.

This study was conducted against Methicillin Resistant *Staphylococcus aureus* MRSA (ATCC 33592) for product Micro-Kill Bleach Germicidal Bleach Solution (Lot# E27S and Lot# E28S). These were tested using ATS Laboratory Protocol No. MDI01051514.GS.6 (copy provided). The product was received as a ready to use liquid and tested as a trigger spray. A loopful of stock slant culture was transferred to an initial 10 mL tube of growth medium, mixed,

and the initial culture was incubated for 24±2 hours at 35-37°C. Following incubation, a 10 µL aliquot of culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1). The final test culture was incubated for 48-54 hours at 35-37°C. The test culture was vortex mixed for 3 to 4 seconds and allowed to stand for ≥10 minutes prior to use. Then the upper portion of the culture was removed, leaving behind any clumps or debris and was pooled in a sterile vessel and mixed. The final test culture was mixed thoroughly prior to use. A 0.10 mL aliquot of FBS was added to 1.90 mL of prepared culture to yield a 5% fetal bovine serum organic soil load. Ten (10) individual sterile glass slide carriers (18 mm x 36 mm) per batch, each in a Petri dish matted with two pieces of filter paper, were inoculated with 10.0 µL of test organism using a calibrated pipettor. Inoculum was uniformly spread over the test surface (approx. 1 square inch) of the slide and covered immediately. This was repeated until all slides were individually inoculated. The culture was vortex mixed periodically during inoculation as necessary. The slides were allowed to dry for 33 minutes at 36.2°C and at 40% relative humidity, appeared visibly dry, and were used within 2 hours of drying. For each lot of test substance, test carriers (in an undisturbed horizontal position) were sprayed with the test substance at staggered intervals at a distance of 6-8 inches using 4 sprays, and sprayed within ±3 seconds of the exposure time following a calibrated timer. Each treated carrier was then held at room temperature (21.0°C) and 56.6% relative humidity for 30 seconds. After exposure, excess liquid was drained off the carrier without touching the carrier to the Petri dish or filter paper. Each carrier was then transferred using sterile forceps following identical staggered intervals to 20 mL aliquots of neutralizing subculture medium (Letheen Broth + 0.1% sodium thiosulfate). The vessel was shaken thoroughly. All subcultures were incubated for 48±2 hours at 35-37°C, and then visually examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation and carrier population.

49. MRID 494865-49, "AOAC Germicidal Spray Method," Test Organism: *Enterobacter cloacae* (ATCC 13047), for product Micro-Kill Bleach Germicidal Bleach Solution, Lot# E27S and Lot# E28S. Study conducted at ATS Labs by Mary J. Miller, M.T. Study completion date – July 9, 2014. Project Number A16854.

This study was conducted against *Enterobacter cloacae* (ATCC 13047). Lot Nos. E27S and E28S of the product, Micro-Kill Bleach Germicidal Bleach Solution, were tested using ATS Laboratory Protocol No. MDI01051714.GS.6 (copy provided). The product was received as a ready to use liquid and tested as a trigger spray. A loop of stock slant culture was transferred to an initial 10 mL tube of growth medium. The tube was mixed and the initial culture was incubated for 24±2 hours at 25-30°C. Following incubation a 10 µL aliquot of culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1). The final test culture was incubated for 48-54 hours at 25-30°C. The test culture was vortex mixed (3-4 seconds) and allowed to stand ≥10 minutes prior to use. The upper portion was removed and pooled in a sterile vessel and mixed. The culture was diluted by combining 1.0 mL of test organism suspension with 4.0 mL of sterile growth medium. The final test culture was mixed thoroughly prior to use. A 0.1 mL aliquot of fetal bovine serum was added to 1.9 mL of prepared culture to achieve a 5% organic soil load. A 10 µL aliquot of the prepared culture was uniformly spread over the individual glass slide carriers (18 mm x 36 mm) per product batch, each in a Petri dish matted with two pieces of filter paper. Inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 35-37°C (36.0°C) and at 40% relative humidity. Carriers were used within 2 hours of drying. Test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface until thoroughly wet (4 full sprays). The carriers were allowed to remain wet for 30 seconds at room temperature (20.8°C) and at 50.9% relative humidity.

Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at identical staggered intervals to 20 mL of Letheen Broth + 0.1% sodium thiosulfate to neutralize. All subcultures were incubated for 48±2 hours at 25-30°C. Subcultures were stored at 2-8°C for 2 days prior to examination. Following incubation and storage, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

50. MRID 494865-50, "AOAC Germicidal Spray Method," Test Organism: *Proteus mirabilis* (ATCC 9240), for product Micro-Kill Bleach Germicidal Bleach Solution, Lot# E27S and Lot# E28S. Study conducted at ATS Labs by Mary J. Miller, M.T. Study completion date – July 10, 2014. Project Number A16855.

This study was conducted against *Proteus mirabilis* (ATCC 9240). Lot Nos. E27S and E28S of the product, Micro-Kill Bleach Germicidal Bleach Solution, were tested using ATS Laboratory Protocol No. MDI01051714.GS.9 (copy provided). The product was received as a ready to use liquid and tested as a trigger spray. A loop of stock slant culture was transferred to an initial 10 mL tube of growth medium. The tube was mixed and the initial culture was incubated for 24±2 hours at 35-37°C. Following incubation a 10 µL aliquot of culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1). The final test culture was incubated for 48-54 hours at 35-37°C. The test culture was vortex mixed (3-4 seconds) and allowed to stand ≥10 minutes prior to use. The upper portion was removed and pooled in a sterile vessel and mixed. The culture was diluted by combining 2.0 mL of test organism suspension with 2.0 mL of sterile growth medium. The final test culture was mixed thoroughly prior to use. A 0.1 mL aliquot of fetal bovine serum was added to 1.9 mL of prepared culture to achieve a 5% organic soil load. A 10 µL aliquot of the prepared culture was uniformly spread over the individual glass slide carriers (18 mm x 36 mm) per product batch, each in a Petri dish matted with two pieces of filter paper. Inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 33 minutes at 25-30°C (27.5-30.0°C) and at 65% relative humidity. Carriers were used within 2 hours of drying. Test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface until thoroughly wet (4 full sprays). The carriers were allowed to remain wet for 30 seconds at room temperature (21.2°C) and at 47.4% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at identical staggered intervals to 20 mL of Letheen Broth + 0.1% sodium thiosulfate to neutralize. All subcultures were incubated for 48±2 hours at 35-37°C. Subcultures were stored at 2-8°C for 2 days prior to examination. Following incubation and storage, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

51. MRID 494865-51, "AOAC Germicidal Spray Method," Test Organism: *Serratia marcescens* (ATCC 14756), for product Micro-Kill Bleach Germicidal Bleach Solution, Lot# E27S and Lot# E28S. Study conducted at ATS Labs by Mary J. Miller. Study completion date – July 1, 2014. Project Number A16856.

This study was conducted against *Serratia marcescens* (ATCC 14756). Lot Nos. E27S and E28S of the product, Micro-Kill Bleach Germicidal Bleach Solution, were tested using ATS Laboratory Protocol No. MDI01051714.GS.10 (copy provided). The product was received as a ready to use liquid and tested as a trigger spray. A loop of stock slant culture was transferred to an initial 10 mL tube of growth medium. The tube was mixed and the initial culture was incubated for 24±2 hours at 35-37°C. Following incubation a 10 µL aliquot of culture was transferred to a 20

x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1). The final test culture was incubated for 48-54 hours at 35-37°C. The test culture was vortex mixed (3-4 seconds) and allowed to stand ≥10 minutes prior to use. The upper portion was removed and pooled in a sterile vessel and mixed. The culture was diluted by combining 1.0 mL of test organism suspension with 2.0 mL of sterile growth medium. The final test culture was mixed thoroughly prior to use. A 0.1 mL aliquot of fetal bovine serum was added to 1.9 mL of prepared culture to achieve a 5% organic soil load. A 10 µL aliquot of the prepared culture was uniformly spread over the individual glass slide carriers (18 mm x 36 mm) per product batch, each in a Petri dish matted with two pieces of filter paper. Inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 35-37°C (36.1°C) and at 52.8% relative humidity. Carriers were used within 2 hours of drying. Test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface until thoroughly wet (4 full sprays). The carriers were allowed to remain wet for 30 seconds at room temperature (21.6°C) and at 53.7% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at identical staggered intervals to 20 mL of Letheen Broth + 0.1% sodium thiosulfate to neutralize. All subcultures were incubated for 48±2 hours at 35-37°C. Subcultures were stored at 2-8°C for 2 days prior to examination. Following incubation and storage, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

52. MRID 494865-52, "Standard Quantitative Disk Carrier Test Method," Test Organism: *Clostridium difficile* – spore form (ATCC 43598) for product Micro-Kill Bleach Germicidal Bleach Solution, Lot# E27S, Lot# E28S, and E29S. Study conducted at ATS Labs by Matthew Sathe. Study completion date – August 11, 2014. Project Number A16882.

This study was conducted against *Clostridium difficile* – spore form (ATCC 43598). Lot Nos. E27S, E28S, and E29S of the product, Micro-Kill Bleach Germicidal Bleach Solution, were tested using ATS Laboratory Protocol No. MDI01052114.QDCT (copy provided). The product was received as a ready to use liquid and tested as a trigger spray. Efficacy could not be determined due to cancellation of the study prior to the generation of valid data. Testing resulted in carrier population control failure and invalid data.

53. MRID 494865-53, "Standard Quantitative Disk Carrier Test Method," Test Organism: *Clostridium difficile* – spore form (ATCC 43598) for product Micro-Kill Bleach Germicidal Bleach Solution, Lot# E27S, Lot# E28S, and E29S. Study conducted at ATS Labs by Matthew Sathe. Study completion date – August 28, 2014. Project Number A17033.

This study was conducted against *Clostridium difficile* – spore form (ATCC 43598). Lot Nos. E27S, E28S, and E29S of the product, Micro-Kill Bleach Germicidal Bleach Solution, were tested using ATS Laboratory Protocol No. MDI01071414.QDCT (copy provided). The product was received as a ready to use liquid and tested as a trigger spray. A stock plate of test organism was used to inoculate 2 pre-reduced 10 mL tubes of Reinforced Clostridial Medium (RCM) using isolated colonies. Each tube was vortex mixed and incubated for 24±2 hours at 35-37°C under anaerobic conditions. Twenty CABA plates were inoculated using the RCM broth culture (100 µL per plate) and incubated for 7-10 days at 36±1°C. The culture was harvested from each plate by adding 5 mL of PBS-T to each plate and gently scraping the surface of each plate with a cell scraper to dislodge the spores. The harvested material was pooled into a sterile 50 mL centrifuge

tube and centrifuged at 1650×g for ~38 minutes. The pellet was resuspended in 20-30 mL PBS-T to wash. The wash step was repeated an additional two times and finally resuspended in 4 mL ST80. The spore suspension was heat treated in a water bath for 10±1 minutes at 65±2°C, cooled to room temperature, and evaluated microscopically. Soil was added to the suspension as indicated in the method. HCl Resistance was determined as indicated in the method. For the contamination of carriers, ten (10.0) µL of culture was placed in the center of each disk using a calibrated positive displacement pipettor. After all disks in each petri dish were inoculated, the dishes were covered and the contaminated carriers were placed in a desiccator containing active desiccant. A vacuum was drawn and the carriers were dried for 30±5 minutes under ambient conditions. The Petri dish lids was removed and the carriers were continued to dry under vacuum for 2 hours at room temperature inside the BSC. The test substance (50 µL) was allowed to remain in contact with the disk for 5 minutes at room temperature (21.1-22.1°C). Following the exposure time, 10.0 mL of neutralizer was added to each vial containing the carriers. The vials containing the carriers were vortex-mixed (this represented the 10^0 dilution). The contents were transferred to separate filter membranes with 0.2 µm porosity. The vials were rinsed with 10 mL of sterile PBS and vortex-mixed once. Each rinse solution was transferred to the same filter membrane. The contents were evacuated, after which each filter membrane was removed aseptically from the filter unit and placed on the surface of an agar plate (BHIY-HT Agar) for recovery of *C. difficile* spores. The subcultures were incubated anaerobically for 72±4 hours at 35-37°C prior to spore enumeration. Controls included those for culture purity, carrier sterility, neutralizer sterility, initial suspension population, neutralization confirmation, carrier population, and HCl resistance.

54. MRID 494865-54, "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces Utilizing Duck Hepatitis B Virus as a Surrogate for Human Hepatitis B Virus," for product Micro-Kill Bleach Germicidal Bleach Solution, Lot# E27S and Lot# E28S. Study conducted at ATS Labs by Shanen Conway. Study completion date – September 30, 2014. Project Number A17193.

This study was conducted against the 10/29/11 strain of Duck Hepatitis B Virus (DHBV) as a Surrogate for Human Hepatitis B Virus, for product Micro-Kill Bleach Germicidal Bleach Solution, Lot# E27S and Lot# E28S. These were tested using ATS Laboratory Protocol No. MDI01082814.DHBV (copy provided). The product was received as a ready to use liquid and tested as a trigger spray. On the day of use, two aliquots of stock virus were thawed, combined, and maintained at a refrigerated temperature until used in the assay. On the day of use, three aliquots of stock virus were thawed, combined and maintained at a refrigerated temperature until used in the assay. The stock virus was adjusted to contain 5% fetal bovine serum in addition to whole duck serum (100% duck serum) as the organic soil load. Indicator Cell cultures of viral free duckling hepatocytes, obtained from VRI Labs, were seeded into sterile twelve well disposable tissue culture labware and were maintained at 36-38°C in a humidified atmosphere at 5-7% CO_2. Test medium used to maintain the cell cultures was Leibovitz L-15 medium supplemented with 0.1% glucose, 10 µM dexamethasone, 10 µg/mL insulin, 20 mM HEPES, 10 µg/mL gentamicin, and 100 units/mL penicillin. Dried virus films were prepared by spreading 200 µL of test virus inoculum uniformly over the bottoms of 6 separate 100 x 15 mm sterile glass petri dishes. These were dried at 20.0°C in a relative humidity of 36.6% for 30 minutes. For each lot of test substance, two dried virus films were exposed for 30 seconds at room temperature (20.0°C) to 4 sprays at a distance of 6-8 inches and held covered for the exposure time. Just prior to the end of the exposure time, the plates were individually scraped with a cell scraper to re-suspend the contents and at the end of the exposure time the virus-test substance mixtures were immediately passed through individual Sephadex columns utilizing the syringe plungers in order to detoxify the mixtures. The filtrates (10^{-1} dilution) were titered by 10-fold serial dilution and each dilution was

then assayed for infectivity and/or cytotoxicity. The Primary duck hepatocyte cells in multiwell culture dishes were inoculated in quadruplicate with 250 µL of the dilutions prepared from test and control groups and were incubated for 9 days at 36-38°C in a humidified atmosphere of 5-7% CO_2 in sterile disposable cell culture labware. On the final day of incubation, the cultures were scored microscopically for cytotoxicity and the cells were fixed with ethanol. An indirect immunofluorescence assay was performed using a monoclonal antibody specific for the envelope protein of the DHBV. Controls included those for input virus control, dried virus control, cytotoxicity, neutralization and cell viability.

V SUMMARY of RESULTS

MRID Number	Organism	Lot #	No. Exhibiting Growth / Total No. Carriers Tested	Carrier Population (Log_{10}CFU/ Carrier)
30 Second Exposure Time				
494865-09	*Staphylococcus aureus* (ATCC 6538)	E27S E28S E29S	0/60 0/60 1/60	5.87
	Pseudomonas aeruginosa (ATCC 15442)	E27S E28S E29S	0/60 0/60 0/60	6.04
	Salmonella enterica (ATCC 10708)	E27S E28S E29S	1/60 0/60 0/60	5.16

MRID Organism	Subculture Medium	Lot #	No. Exhibiting Growth / Total No. Carriers Tested	Carrier Population (Log_{10}CFU/ Carrier)
3 Minute Exposure Time				
494865-10 *Mycobacterium bovis* (BCG)	Modified Proskauer-Beck Broth	E27S E28S	10/10 10/10	5.05
	Middlebrook 7H9 Broth	E27S E28S	10/10 10/10	
	Kirchner's Medium	E27S E28S	10/10 10/10	

MRID Number	Organism	Lot #	No. Exhibiting Growth / Total No. Carriers Tested	Carrier Population Log_{10}CFU/ Carrier
1-Minute Exposure Time				
494865-11	*Candida albicans* (ATCC 10231)	E27S	0/10	5.64
		E28S	0/10	
494865-12	*Trichophyton mentagrophytes* (ATCC 9533)	E27S	0/10	5.64
		E28S	0/10	
494865-34	*Aspergillus brasiliensis* (ATCC 16404)	E27S	10/10	5.69
		E28S	10/10	

MRID Number	Organism	Lot #	No. Exhibiting Growth / Total No. Carriers Tested	Carrier Population (Log_{10}CFU/ Carrier)
30 Second Exposure Time				
494865-23	*Shigella dysenteriae* (ATCC 11835)	E27S E28S	0/10 0/10	5.13
494865-24	*Listeria monocytogenes* (ATCC 19117)	E27S E28S	0/10 0/10	5.44
494865-25	New Delhi metallo-beta-lactamase 1 (NDM-1) positive *Klebsiella pneumoniae* (CDC 1000527)	E27S E28S	0/10 0/10	4.72
494865-26	Multi-Drug Resistant (MDR) *Acinetobacter baumannii* (ATCC 19606)	E27S E28S	0/10 0/10	5.79
494865-27	Vancomycin Resistant *Enterococcus faecalis* – VRE (ATCC 51575)	E27S E28S	0/10 0/10	5.64
494865-28	*Streptococcus pneumoniae* (ATCC 6305)	E27S E28S	0/10 0/10	4.50
494865-29	*Legionella pneumophila* (ATCC 33153)	E27S E28S	0/10 0/10	5.96
494865-30	Linezolid Resistant *Staphylococcus aureus* (LRS 119)	E27S E28S	0/10 0/10	5.57
494865-31	Vancomycin Intermediate Resistant *Staphylococcus aureus* -VISA (HIP 5836)	E27S E28S	0/10 0/10	6.10
494865-32	*Bordetella pertussis* (ATCC 12743)	E27S E28S	0/10 0/10	5.53
494865-33	*Campylobacter jejuni* (ATCC 29428)	E27S E28S	0/10 0/10	6.15
494865-35	Extended-Spectrum beta-lactamase (ESBL) positive *Escherichia coli* (ATCC BAA-196)	E27S E28S	0/10 0/10	6.18
494865-36	*Escherichia coli* (ATCC 11229)	E27S E28S	0/10 0/10	5.25
494865-37	Extended-Spectrum beta-lactamase (ESBL) positive *Klebsiella pneumoniae* (ATCC 700603)	E27S E28S	0/10 0/10	4.94

MRID Number	Organism	Lot #	No. Exhibiting Growth / Total No. Carriers Tested	Carrier Population (Log_{10}CFU/ Carrier)
30 Second Exposure Time				
494865-38	Multi-Drug Resistant (MDR) *Enterococcus faecium* (ATCC 51559)	E27S E28S	0/10 0/10	6.17
494865-39	Community Acquired Methicillin Resistant *Staphylococcus aureus* -CA-MRSA Genotype USA 300 (NRS 384)	E27S E28S	1/10 0/10	6.33
494865-40	Community Acquired Methicillin Resistant *Staphylococcus aureus* -CA-MRSA Genotype USA 400 (NRS 123)	E27S E28S	0/10 0/10	6.22
494865-41	*Escherichia coli* O157:H7 (ATCC 35150)	E27S E28S	0/10 0/10	5.98
494865-42	Vancomycin Resistant *Staphylococcus aureus* -VRSA (VRS1)	E27S E28S	0/10 0/10	6.23
494865-43	Carbapenem Resistant *Klebsiella pneumoniae* (ATCC BAA-1705)	E27S E28S	0/10 0/10	5.15
494865-44	New Delhi metallo-beta-lactamase 1 (NDM-1) positive *Escherichia coli* (CDC 1001728)	E27S E28S	0/10 0/10	5.36
494865-45	*Streptococcus pyogenes* (ATCC 19615)	E27S E28S	0/10 0/10	7.45
494865-46	*Klebsiella pneumoniae* (ATCC 4352)	E27S E28S	0/10 0/10	5.71
494865-47	*Enterobacter aerogenes* (ATCC 13048)	E27S E28S	0/10 0/10	6.54
494865-48	Methicillin Resistant *Staphylococcus aureus* MRSA (ATCC 33592)	E27S E28S	0/10 0/10	6.56
494865-49	*Enterobacter cloacae* (ATCC 13047)	E27S E28S	0/10 0/10	6.03
494865-50	*Proteus mirabilis* (ATCC 9240)	E27S E28S	0/10 0/10	5.88
494865-51	*Serratia marcescens* (ATCC 14756)	E27S E28S	0/10 0/10	6.64

MRID Number	Organism	Results			Dried Virus Count
30 Second Exposure Time					
494865-01	Rotavirus, Strain WA		Lot# E27S	Lot# E28S	
		10^{-1} to 10^{-7} Dilutions	Complete inactivation	Complete inactivation	$10^{6.75}$ $TCID_{50}$/ 100 µL
		$TCID_{50}$/100 µL	$\leq 10^{0.50}$	$\leq 10^{0.50}$	
494865-02	Respiratory Syncytial Virus (ATCC VR-26, Strain Long)	10^{-2} to 10^{-7} Dilutions	Complete inactivation	Complete inactivation	$10^{5.25}$ $TCID_{50}$/ 100 µL
		$TCID_{50}$/100 µL	$\leq 10^{1.50}$	$\leq 10^{1.50}$	
494865-03	Rhinovirus type 37 (ATCC VR-1147, Strain 151-1)	10^{-1} to 10^{-6} Dilutions	Complete inactivation	Complete inactivation	$10^{6.00}$ $TCID_{50}$/ 100 µL
		$TCID_{50}$/100 µL	$\leq 10^{0.50}$	$\leq 10^{0.50}$	
494865-04	Adenovirus type 5, ATCC VR-5, Strain Adenoid 75	10^{-1} to 10^{-9} Dilutions	Complete inactivation	Complete inactivation	$10^{7.75}$ $TCID_{50}$/ 100 µL
		$TCID_{50}$/100 µL	$\leq 10^{0.50}$	$\leq 10^{0.50}$	
494865-05	Poliovirus type 1, ATCC VR-1562, Strain Chat	10^{-1} to 10^{-6} Dilutions	Complete inactivation	Complete inactivation	$10^{4.50}$ $TCID_{50}$/ 100 µL
		$TCID_{50}$/100 µL	$\leq 10^{0.50}$	$\leq 10^{0.50}$	
494865-06	Feline Panleukopenia virus, ATCC VR-648, Strain Philips-Roxane	10^{-1} to 10^{-6} Dilutions	Complete inactivation	Complete inactivation	$10^{4.50}$ $TCID_{50}$/ 100 µL
		$TCID_{50}$/100 µL	$\leq 10^{0.50}$	$\leq 10^{0.50}$	
494865-07	Hepatitis A virus Strain HM-175	10^{-1} to 10^{-7} Dilutions	Complete inactivation	Complete inactivation	$10^{5.50}$ $TCID_{50}$/ 100 µL
		$TCID_{50}$/100 µL	$\leq 10^{0.50}$	$\leq 10^{0.50}$	
494865-08	Canine Parvovirus Strain Cornell-780916-80 (ATCC VR-2017)	10^{-1} to 10^{-6} Dilutions	Complete inactivation	Complete inactivation	$10^{5.50}$ $TCID_{50}$/ 100 µL
		$TCID_{50}$/100 µL	$\leq 10^{0.50}$	$\leq 10^{0.50}$	
494865-13	Influenza A virus, Hong Kong strain (ATCC VR-544)	10^{-1} to 10^{-8} Dilutions	Complete inactivation	Complete inactivation	$10^{7.25}$ $TCID_{50}$/ 100 µL
		$TCID_{50}$/100 µL	$\leq 10^{0.50}$	$\leq 10^{0.50}$	
494865-14	Human Immunodeficiency Virus type 1 (Strain HTLV-III_B)	10^{-2} to 10^{-7} Dilutions	Complete inactivation	Complete inactivation	$10^{5.25}$ $TCID_{50}$/200 µL
		$TCID_{50}$/200 µL	$\leq 10^{1.50}$	$\leq 10^{1.50}$	

MRID Number	Organism	Results			Dried Virus Count
30 Second Exposure Time					
494865-15	Human Coronavirus, ATCC VR-740, Strain 229E		Lot# E27S	Lot# E28S	
		10^{-2} to 10^{-7} Dilutions	Complete inactivation	Complete inactivation	$10^{5.50}$ $TCID_{50}$/ 100 µL
		$TCID_{50}$/100 µL	$\leq 10^{1.50}$	$\leq 10^{1.50}$	
494865-16	Feline Calicivirus (F-9 strain, ATCC VR-782)*	10^{-1} to 10^{-4} Dilutions	Complete inactivation	Complete inactivation	$10^{7.75}$ $TCID_{50}$/ 100 µL
		$TCID_{50}$/100 µL	$\leq 10^{0.50}$	$\leq 10^{0.50}$	
494865-17	Oregon C24v-genotype 1 strain of Bovine Viral Diarrhea Virus (BVDV)**	10^{-1} to 10^{-4} Dilutions	Complete inactivation	Complete inactivation	$10^{5.38}$ $TCID_{50}$/ 100 µL
		$TCID_{50}$/100 µL	$\leq 10^{0.50}$	$\leq 10^{0.50}$	
494865-18	Avian Influenza A (H7N9) virus (CDC # 2013759189, Strain wildtype A/Anhui/1/2013)	10^{-1} to 10^{-8} Dilutions	Complete inactivation	Complete inactivation	$10^{7.00}$ $TCID_{50}$/ 100 µL
		$TCID_{50}$/100 µL	$\leq 10^{0.50}$	$\leq 10^{0.50}$	
494865-19	Avian Influenza A (H5N1) virus (Strain VNH5N1-PR8/CDC-RG, CDC # 2006719965)	10^{-1} to 10^{-8} Dilutions	Complete inactivation	Complete inactivation	$10^{6.50}$ $TCID_{50}$/ 100 µL
		$TCID_{50}$/100 µL	$\leq 10^{0.50}$	$\leq 10^{0.50}$	
494865-20	Avian Influenza A virus (H3N2) Reassortant virus, Strain A/Washington/897/80 x A/Mallard/New York/6750/78, ATCC VR-2072	10^{-1} to 10^{-8} Dilutions	Complete inactivation	Complete inactivation	$10^{5.75}$ $TCID_{50}$/ 100 µL
		$TCID_{50}$/100 µL	$\leq 10^{0.50}$	$\leq 10^{0.50}$	
494865-21	2009-H1N1 Influenza A virus (Novel H1N1), Strain A/Mexico/4108/2009 (CDC #2009712192)	10^{-1} to 10^{-8} Dilutions	Complete inactivation	Complete inactivation	$10^{6.25}$ $TCID_{50}$/ 100 µL
		$TCID_{50}$/100 µL	$\leq 10^{0.50}$	$\leq 10^{0.50}$	
494865-22	Herpes simplex virus type 2, ATCC VR-734, Strain G	10^{-1} to 10^{-7} Dilutions	Complete inactivation	Complete inactivation	$10^{5.75}$ $TCID_{50}$/ 100 µL
		$TCID_{50}$/100 µL	$\leq 10^{0.50}$	$\leq 10^{0.50}$	
494865-54	10/29/11 strain of Duck Hepatitis B Virus (DHBV)***	10^{-1} to 10^{-4} Dilutions	Complete inactivation	Complete inactivation	$10^{5.63}$ $TCID_{50}$/ 250 µL
		$TCID_{50}$/250 µL	$\leq 10^{0.50}$	$\leq 10^{0.50}$	

* as a Surrogate for Norovirus
** as a Surrogate for Human Hepatitis C Virus
*** as a Surrogate for Human Hepatitis B Virus

MRID Number	Organism	Lot No.	Mean Log_{10} CFU/Carrier (LD) Recovered	Mean Log_{10} CFU/Carrier (LD) Initially Present	Log Reduction
5 minute exposure time					
494865-53	*Clostridium difficile*- spore form (ATCC 43598)	E27S	<1.00	6.33	≥6.33
		E28S	<1.00	6.15	≥6.15
		E29S	<1.00	6.29	≥6.29

VI CONCLUSIONS

1. The submitted efficacy data **support** the ready–to-use spray product as a disinfectant against the following bacteria on hard, non-porous non-food contact surfaces with a 5% organic soil load for a 30 second contact time at room temperature:

Staphylococcus aureus (ATCC 6538)	494865-09
Pseudomonas aeruginosa (ATCC 15442)	494865-09
Salmonella enterica (ATCC 10708)	494865-09

Acceptable killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. Neutralization confirmation testing showed positive growth of the microorganisms. Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth.

2. The submitted efficacy data **support** the ready–to-use spray product as a disinfectant against the following additional bacteria on hard, non-porous non-food contact surfaces with a 5% organic soil load for a 30 second contact time at room temperature:

Shigella dysenteriae (ATCC 11835)	494865-23
Listeria monocytogenes (ATCC 19117)	494865-24
New Delhi metallo-beta-lactamase 1 (NDM-1) positive *Klebsiella pneumoniae* (CDC 1000527)	494865-25
Multi-Drug Resistant (MDR) *Acinetobacter baumannii* (ATCC 19606)	494865-26
Vancomycin Resistant *Enterococcus faecalis* – VRE (ATCC 51575)	494865-27
Streptococcus pneumoniae (ATCC 6305)	494865-28
Legionella pneumophila (ATCC 33153)	494865-29
Linezolid Resistant *Staphylococcus aureus* (LRS 119)	494865-30
Vancomycin Intermediate Resistant *Staphylococcus aureus* -VISA (HIP 5836)	494865-31
Bordetella pertussis (ATCC 12743)	494865-32
Campylobacter jejuni (ATCC 29428)	494865-33
Extended-Spectrum beta-lactamase (ESBL) positive *Escherichia coli* (ATCC BAA-196)	494865-35
Escherichia coli (ATCC 11229)	494865-36
Extended-Spectrum beta-lactamase (ESBL) positive *Klebsiella pneumoniae* (ATCC 700603)	494865-37
Multi-Drug Resistant (MDR) *Enterococcus faecium* (ATCC 51559)	494865-38
Community Acquired Methicillin Resistant *Staphylococcus aureus* -CA-MRSA Genotype USA 400 (NRS 123)	494865-40
Escherichia coli O157:H7 (ATCC 35150)	494865-41
Vancomycin Resistant *Staphylococcus aureus* -VRSA (VRS1)	494865-42
Carbapenem Resistant *Klebsiella pneumoniae* (ATCC BAA-1705)	494865-43
New Delhi metallo-beta-lactamase 1 (NDM-1) positive *Escherichia coli* (CDC 1001728)	494865-44
Streptococcus pyogenes (ATCC 19615)	494865-45
Klebsiella pneumoniae (ATCC 4352)	494865-46
Enterobacter aerogenes (ATCC 13048)	494865-47
Methicillin Resistant *Staphylococcus aureus* MRSA (ATCC 33592)	494865-48
Enterobacter cloacae (ATCC 13047)	494865-49
Proteus mirabilis (ATCC 9240)	494865-50
Serratia marcescens (ATCC 14756)	494865-51

Acceptable killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. Neutralization confirmation testing showed positive growth of the microorganisms. Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth.

3. The submitted efficacy data **support** the ready–to-use spray product as a disinfectant against the following fungi on hard, non-porous non-food contact surfaces with a 5% organic soil load for a 1-minute contact time at room temperature:

Trichophyton mentagrophytes, (ATCC 9533)	494865-12
Candida albicans (ATCC 10231)	494865-11

Acceptable killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. Neutralization confirmation testing showed positive growth of the microorganisms. Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth.

4. The submitted efficacy data **support** the ready–to-use spray product as a disinfectant against the following viruses on hard, non-porous non- food contact surfaces with a 5% organic soil load for a 30 second contact time at room temperature:

Rotavirus, Strain WA	494865-01
Respiratory Syncytial Virus (ATCC VR-26, Strain Long)	494865-02
Rhinovirus type 37 (ATCC VR-1147, Strain 151-1)	494865-03
Adenovirus type 5, ATCC VR-5, Strain Adenoid 75	494865-04
Poliovirus type 1, ATCC VR-1562, Strain Chat	494865-05
Feline Panleukopenia virus, ATCC VR-648, Strain Philips-Roxane	494865-06
Hepatitis A virus Strain HM-175	494865-07
Canine Parvovirus Strain Cornell-780916-80 (ATCC VR-2017)	494865-08
Influenza A virus, Hong Kong strain (ATCC VR-544)	494865-13
Human Immunodeficiency Virus type 1 (Strain HTLV-III_B)	494865-14
Human Coronavirus, ATCC VR-740, Strain 229E	494865-15
Feline Calicivirus (F-9 strain, ATCC VR-782) as a Surrogate for Norovirus	494865-16
Oregon C24v-genotype 1 strain of Bovine Viral Diarrhea Virus (BVDV) as a Surrogate for Human Hepatitis C Virus	494865-17
Avian Influenza A (H7N9) virus (CDC # 2013759189, Strain wildtype A/Anhui/1/2013)	494865-18
Avian Influenza A (H5N1) virus (Strain VNH5N1-PR8/CDC-RG, CDC # 2006719965)	494865-19
Avian Influenza A virus (H3N2) Reassortant virus, Strain A/Washington/897/80 x A/Mallard/New York/6750/78, ATCC VR-2072	494865-20
2009-H1N1 Influenza A virus (Novel H1N1), Strain A/Mexico/4108/2009 (CDC #2009712192)	494865-21
Herpes simplex virus type 2, ATCC VR-734, Strain G	494865-22
10/29/11 strain of Duck Hepatitis B Virus (DHBV) as a Surrogate for Human Hepatitis B Virus	494865-54

Recoverable virus titers of at least 10^4 were achieved. Complete inactivation (no growth) was indicated in all dilutions tested. At least a 3-log reduction in titer was demonstrated beyond the cytotoxic level.

5. The submitted efficacy data **support** the use of the ready-to-use spray product as a disinfectant against spores of the following bacteria on hard, non-porous surfaces for an 5 minute contact time at room temperature:

Clostridium difficile (ATCC 43598) 494865-53

Acceptable killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. Neutralization confirmation testing showed positive growth of the microorganisms. Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth. Acceptable carrier counts were achieved. A greater than six log reduction in spore challenge was achieved for all three test lots evaluated.

6. The submitted efficacy data **do not support** the ready–to-use spray product as a disinfectant against the following bacterium on hard, non-porous non-food contact surfaces with a 5% organic soil load for a 30 second contact time at room temperature:

Community Acquired Methicillin Resistant
Staphylococcus aureus -CA-MRSA Genotype USA 300 (NRS 384) 494865-39

Acceptable killing was **not observed** in the subcultures of the required number of carriers tested against the required number of product lots.

7. The submitted efficacy data **do not support** the ready–to-use spray product as a disinfectant against the following fungi on hard, non-porous non-food contact surfaces with a 5% organic soil load for a 1-minute contact time at room temperature:

Aspergillus brasiliensis (ATCC 16404) 494865-34

Acceptable killing was **not observed** in the subcultures of the required number of carriers tested against the required number of product lots.

8. The submitted efficacy data **do not support** the ready–to-use spray product as a disinfectant against the following mycobacterium on hard, non-porous surfaces with a 5% organic soil load for a 3-minute contact time at room temperature:

Mycobacterium bovis – BCG 494865-10

Acceptable killing was **not observed** in the subcultures of the required number of carriers tested against the required number of product lots.

VII RECOMMENDATIONS

1. The label claims that the ready-to-use spray product is a disinfectant against the following bacteria on hard, non-porous non- food contact surfaces for a 30 second contact time in the presence of 5% serum load:

Staphylococcus aureus (ATCC 6538)
Pseudomonas aeruginosa (ATCC 15442)
Salmonella enterica (ATCC 10708)
Shigella dysenteriae (ATCC 11835)
Listeria monocytogenes (ATCC 19117)
New Delhi metallo-beta-lactamase 1 (NDM-1) positive *Klebsiella pneumoniae* (CDC 1000527)
Multi-Drug Resistant (MDR) *Acinetobacter baumannii* (ATCC 19606)
Vancomycin Resistant *Enterococcus faecalis* – VRE (ATCC 51575)
Streptococcus pneumoniae (ATCC 6305)
Legionella pneumophila (ATCC 33153)
Linezolid Resistant *Staphylococcus aureus* (LRS 119)
Vancomycin Intermediate Resistant *Staphylococcus aureus* -VISA (HIP 5836)
Bordetella pertussis (ATCC 12743)
Campylobacter jejuni (ATCC 29428)
Extended-Spectrum beta-lactamase (ESBL) positive *Escherichia coli* (ATCC BAA-196)
Escherichia coli (ATCC 11229)
Extended-Spectrum beta-lactamase (ESBL) positive *Klebsiella pneumoniae* (ATCC 700603)
Multi-Drug Resistant (MDR) *Enterococcus faecium* (ATCC 51559)
Community Acquired Methicillin Resistant *Staphylococcus aureus*
-CA-MRSA Genotype USA 400 (NRS 123)
Escherichia coli O157:H7 (ATCC 35150)
Vancomycin Resistant *Staphylococcus aureus* -VRSA (VRS1)
Carbapenem Resistant *Klebsiella pneumoniae* (ATCC BAA-1705)
New Delhi metallo-beta-lactamase 1 (NDM-1) positive *Escherichia coli* (CDC 1001728)
Streptococcus pyogenes (ATCC 19615)
Klebsiella pneumoniae (ATCC 4352)
Enterobacter aerogenes (ATCC 13048)
Methicillin Resistant *Staphylococcus aureus* MRSA (ATCC 33592)
Enterobacter cloacae (ATCC 13047)
Proteus mirabilis (ATCC 9240)
Serratia marcescens (ATCC 14756)

These claims are acceptable as they are supported by the submitted data.

2. The label claims the ready–to-use product is a disinfectant against the following fungi on hard, non-porous non-food contact surfaces for a contact time of 1-minute in the presence of 5% serum load:

Trichophyton mentagrophytes, (ATCC 9533)
Candida albicans (ATCC 10231)

These claims are acceptable as they are supported by the submitted data.

3. The label claims that the ready-to-use product is a disinfectant against the following viruses on hard, non-porous non- food contact surfaces for a 30 second contact time in the presence of 5% serum load:

Rotavirus, Strain WA

Respiratory Syncytial Virus (ATCC VR-26, Strain Long)
Rhinovirus type 37 (ATCC VR-1147, Strain 151-1)
Adenovirus type 5, ATCC VR-5, Strain Adenoid 75
Poliovirus type 1, ATCC VR-1562, Strain Chat
Feline Panleukopenia virus, ATCC VR-648, Strain Philips-Roxane
Hepatitis A virus Strain HM-175
Canine Parvovirus Strain Cornell-780916-80 (ATCC VR-2017)
Influenza A virus, Hong Kong strain (ATCC VR-544)
Human Immunodeficiency Virus type 1 (Strain HTLV-III_B)
Human Coronavirus, ATCC VR-740, Strain 229E
Feline Calicivirus (F-9 strain, ATCC VR-782) as a Surrogate for Norovirus
Oregon C24v-genotype 1 strain of Bovine Viral Diarrhea Virus (BVDV)
as a Surrogate for Human Hepatitis C Virus
Avian Influenza A (H7N9) virus (CDC # 2013759189, Strain wildtype A/Anhui/1/2013)
Avian Influenza A (H5N1) virus (Strain VNH5N1-PR8/CDC-RG, CDC # 2006719965)
Avian Influenza A virus (H3N2) Reassortant virus, Strain A/Washington/897/80 x
A/Mallard/New York/6750/78, ATCC VR-2072
2009-H1N1 Influenza A virus (Novel H1N1), Strain A/Mexico/4108/2009 (CDC #2009712192)
Herpes simplex virus type 2, ATCC VR-734, Strain G
10/29/11 strain of Duck Hepatitis B Virus (DHBV) as a Surrogate for Human Hepatitis B Virus

These claims are acceptable as they are supported by the submitted data.

4. The label claims that ready-to-use spray is a disinfectant against *Clostridium difficile* – spore form (ATCC 43598) on hard, non-porous surfaces for a 5-minute contact time.

This claim is acceptable and is supported by the submitted efficacy data.

5. The label claims that the ready-to-use spray product is a disinfectant against the following bacteria on hard, non-porous non- food contact surfaces for a 30 second contact time in the presence of 5% serum load:

Community Acquired Methicillin Resistant *Staphylococcus aureus* -CA-MRSA Genotype USA 300 (NRS 384)

This claim is NOT acceptable and is NOT supported by the submitted efficacy data.

6. The label claims the ready–to-use product is a disinfectant against the following fungi on hard, non-porous non-food contact surfaces for a contact time of 1 minute in the presence of 5% serum load:

Aspergillus brasiliensis (ATCC 16404)

This claim is NOT acceptable and is NOT supported by the submitted efficacy data.

7. The label claims the ready–to-use spray product as a disinfectant with tuberculocidal activity against *Mycobacterium bovis* – BCG on hard, non-porous surfaces with a 5% organic soil load for a 3-minute contact time at room temperature:

This claim is NOT acceptable and is NOT supported by the submitted efficacy data.

LABEL RECOMMENDATIONS

- When describing 1:10 dilution, include reference to household bleach in all cases.
- Pg. 5: Change 99.9999% to 99.9%.
- Pg. 7: Remove bullet #4. It falsely implies the product must be diluted 1:10 before use.
- Pg. 7: Remove statement, "Based on Federal EPA master labels and state registration reviews as of [date of most recently approved label].
- Pg. 7: Tenth bullet, remove blood pressure cuffs from list of use sites, this is not a hard non-porous surface.
- Pg. 8: Qualify infectious organisms in statement, "Contains sodium hypochlorite in a 1:10 dilution that is recommended by CDC guidelines for effective disinfecting against infectious organisms.
- Pg. 9 (above 1st bullet) and 11 (1st bullet): Remove "privacy curtains" from list of use sites, this is not a hard non-porous surface.
- Pg. 9: Statement, "Kills 99.99% of germs," change to 99.9%.
- Pg. 10: Remove bullet with reference to 42 CFR 483.65.
- Multiple instances throughout the label: Qualify ALL references to the product being "sporicidal" and "virucidal" to specify *C. difficile* spores and the listing of viruses (i.e., by use of an asterisk) respectively.
- Pg. 13: Bullet #4 – remove reference to the product as a sanitizer.
- Multiple instances throughout the label: Remove any and all references to EPA, CDC, OSHA, and FDA regulations/recommendations/guidelines etc.
- Multiple instances throughout the label: Remove references Federal and/or State regulations/recommendations/guidelines etc.
- Use Directions for treatment of *C. difficile* spores: Add instructions for a pre-cleaning step.
- Multiple instances throughout the label: Qualify or remove the generalized statements indicating "one-step" and/or "no pre-cleaning." Treatment of *C. difficile* spores requires a pre-cleaning step so broad references such as [Pg. 8 of 15, bullet #4] are false and/or misleading unless excepting treatment of *C. difficile* spores.